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THE INFECTION OF RABBITS WITH THE TUBERCLE BACILLUS BY WAY OF THE TRACHEA

STUDIES ON THE DEFENSIVE AND METABOLIC APPARATUS OF THE LUNGS *

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BOSTON

The aerogenous infection of animals with the tubercle bacillus is of interest for two reasons: (1) Because of the scantiness of experimental studies of this particular mode of infection with Koch's bacillus. This dearth is the most surprising since the inhalation of the tubercle bacillus is regarded by most observers as being the essential way of infection in man. (2). In connection with the recent views of the pulmonary structure. From previous studies [1] it was inferred that the inflammatory reaction in pathologic conditions of the lungs originates in situ from the cells lying without (i. e., lining) and within the alveolar septums, and also from the peribronchial and the perivascular cells. It then appeared that the cells commonly designated as "alveolar epithelium" are to all appearances not epithelial, but of mesenchymal origin.

This conception, while accepted by one group of observers, was contradicted by another. The studies with the tubercle bacillus were then undertaken in order to find new evidences pertaining to this theory. For, as Maeterlinck [2] expressed it: "To the objection based on an experiment, the best reply of all must be a counter-experiment."

MATERIALS AND METHODS

The experiments were conducted on full-grown rabbits. A 6 weeks old culture of bovine tubercle bacilli grown on glycerine agar, secured from the American Type Culture Collection, was used. In each case the trachea of the animal was

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* From the Surgical Laboratory of the Peter Bent Brigham Hospital and the Laboratory for Surgical Research, Harvard Medical School.

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* This work was made possible through the assistance of Prof. Harvey Cushing.

1. Fried, B. M.: The Origin of Histiocytes (Macrophages) in the Lungs, Arch. Path. **3**:751, 1927; The Defensive and Metabolic Apparatus of the Lung; The Lungs and the Macrophage System, ibid. **6**:1008, 1928; The Infection of Rabbits with the Anthrax Bacillus by Way of the Trachea, ibid. **10**:213, 1930.

2. Maeterlinck, Maurice: La vie des abeilles, Paris, Fasquelle.

exposed in a manner described previously,[3] and 0.5 mg. of the acid-fast bacilli emulsified in 1 cc. of a physiologic solution of sodium chloride was injected into the lung via the trachea.

The animals were killed by an air embolus at intervals of from one minute to several weeks after the intratracheal injections.

Tissues were fixed in a 10 per cent solution of formaldehyde, in Zenker's fluid and in alcohol. Paraffin and frozen sections were stained with hematoxylin and eosin, with methylene blue and eosin and with Ziehl-Neelsen's stain for acid-fast bacilli.

OBSERVATIONS

After One Minute.—When the lungs were removed from the thoracic cavity about one minute after the intratracheal injection of the tubercle bacilli, they

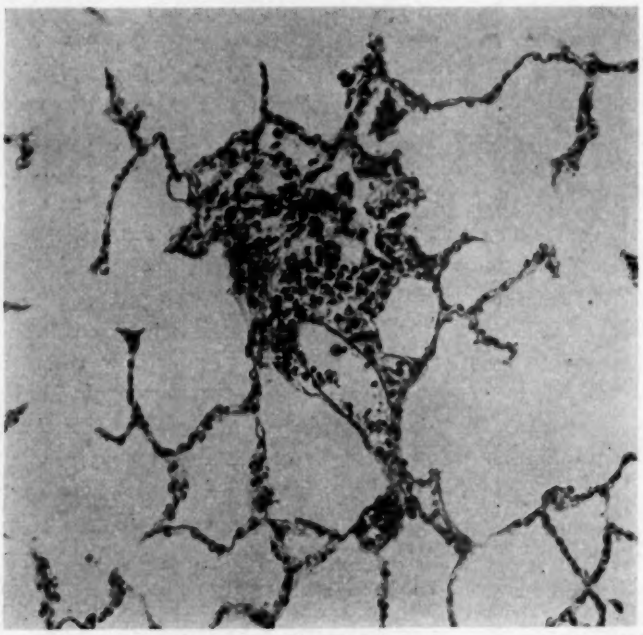

Fig. 1.—The reaction found in the pulmonary alveolus of the rabbit one minute after intratracheal injection of bovine tubercle bacilli. Methylene blue and eosin; × 175.

showed no gross changes. However, sections studied with the microscope revealed an early remarkable response of the pulmonary tissue as follows:

Just as in the experiments with vital staining, in which the dye impregnated the lung in patches only, so the tubercle bacilli produced multiple foci of lesions, which under the lenses of low power were conspicuous as groups of cells lying close to the septums (figs. 1, 2 and 3). The alveoli, which vary in shape and size, could be easily made out, showing moderately distended septal capillaries containing erythrocytes arranged in one single file. With the high-power lenses the following details were remarkable (figs. 2 and 3): Most of the cells that had so rapidly

3. Fried, B. M., and Proctor, E. E.: The Appearance of Specific Antibodies in the Serum of Rabbits by Intratracheal and Intravenous Injections of Living Tubercle Bacillus, Proc. Soc. Exper. Biol. & Med. **21**:396, 1924.

proliferated remained in close connection with the wall of the air sac. Indeed, they did not represent a continuous membrane, but were discontinuous, forming agglomerations here and there. A few cells were seen to lie free in the alveolar lumen, and once in a great while the liberated cells were in mitosis, whereas amitosis could be seen in the sessile cells only.

The cells varied in size from that of a medium-sized lymphocyte to that of a voluminous phagocyte. In the smaller cells the ill defined cytoplasm was barely visible, and the nuclei were irregularly round and contained coarse chromatin

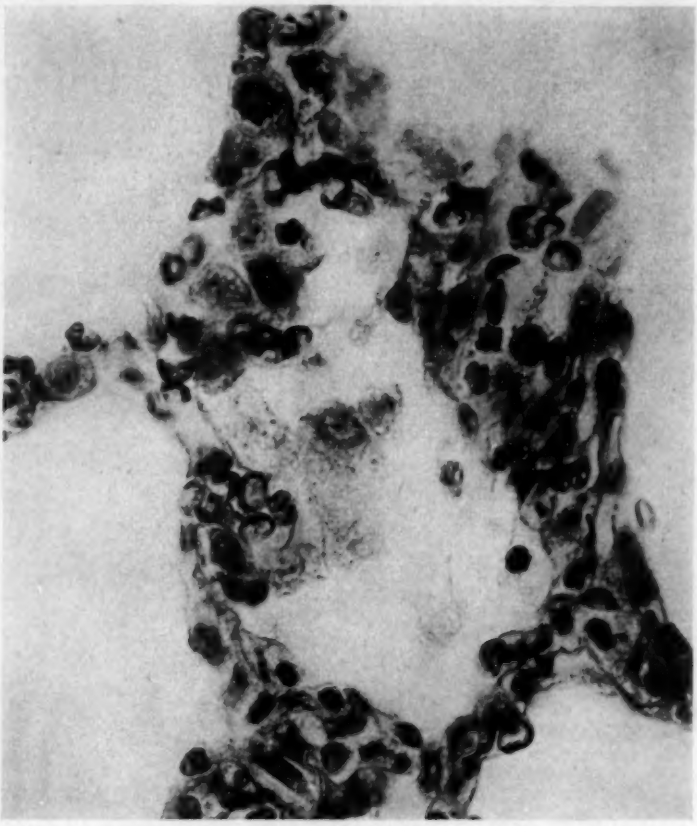

Fig. 2.—The morphology of the proliferated cells lining the air sac of the rabbit one minute after aerogenous infection with Koch's bacillus. Mitotic figures are seen in the upper segment of the photomicrograph. Methylene blue and eosin; × 850.

particles. There were numerous transitions between the small and the large varieties of cells, this being shown by variations in the size of the cytoplasm, but not by the nucleus, which remained practically unchanged. In the large cells the cytoplasm was irregular and foamy, being two or three times larger than the nucleus, which was pushed to the cellular periphery.

The cells that "stuck" to the wall of the air vesicle occasionally contained an acid-fast bacillus in their cytoplasm, while those lying free were often loaded with Koch's bacilli.

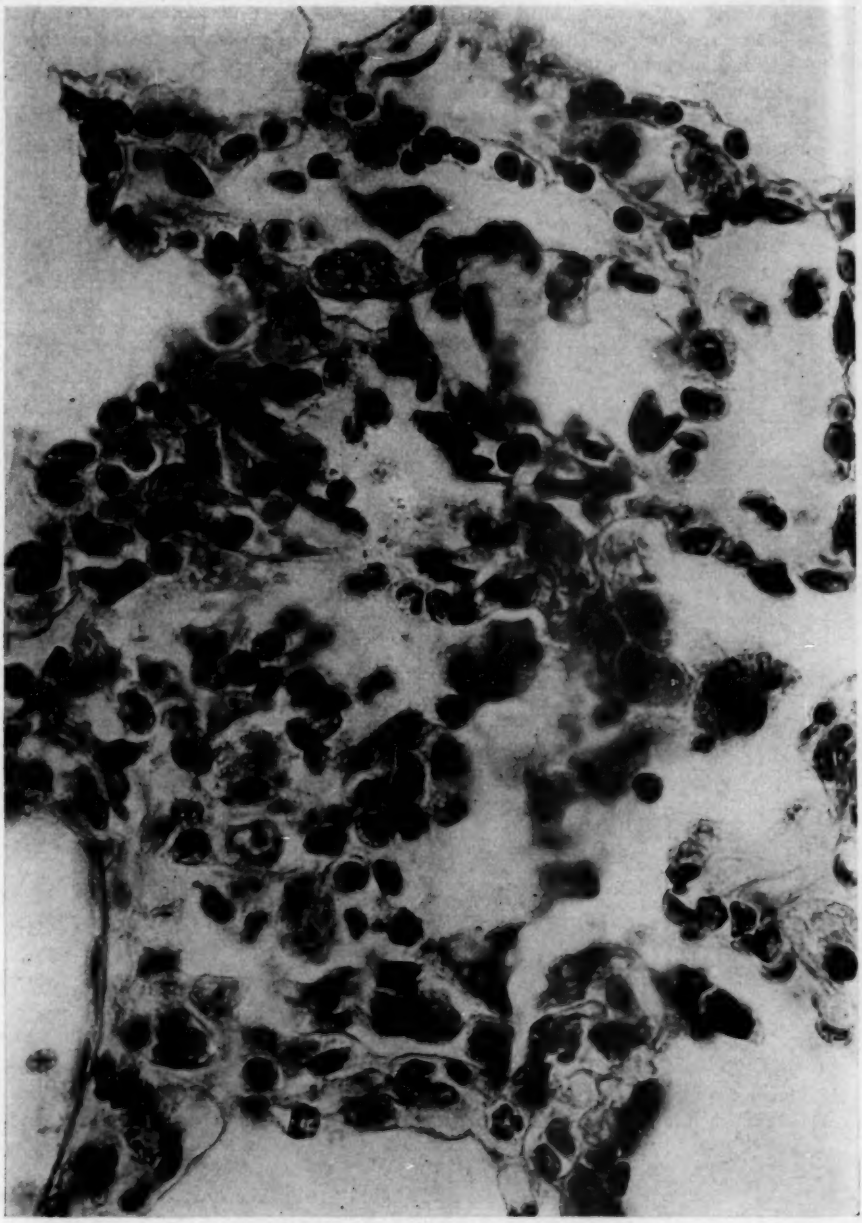

Fig. 3.—A section from the lung of a rabbit that died one minute after intratracheal injection of acid-fast bacilli. The morphology of the fixed and of the wandering cells should be noticed. Methylene blue and eosin; × 380.

Granulocytes could be seen rarely between the groups of large mononuclear cells. An occasional one contained a tubercle bacillus, and sometimes a polymorphonuclear white cell could be found to be, in its turn, phagocytosed by a large mononuclear cell.

The alveolar lumen contained no serum, fibrin or red cells. The endothelium of the septal capillaries showed no changes and no phagocytosis. Likewise, no changes were found in the large blood vessels.

However, the reaction to the tubercle bacilli appeared microscopically to be focal; a search revealed that "irregularities" were also conspicuous in the vicinity

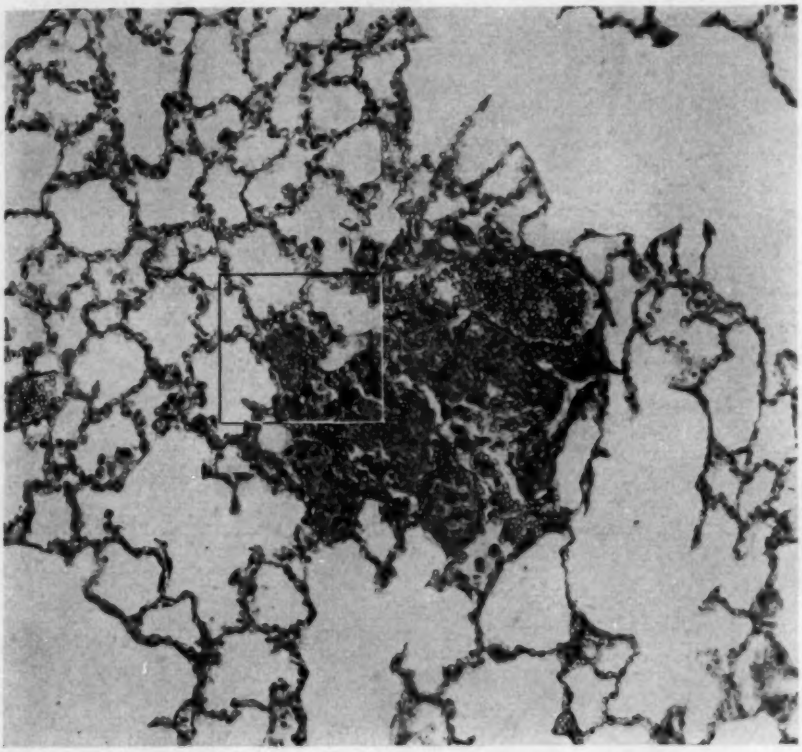

Fig. 4.—The aspect of a "miliary granule" formed in the rabbit's lung five minutes after injection of tubercle bacilli via the trachea. The proliferated alveolar "epithelial" cells are transformed into epithelioid cells (parenchymatous alveolitis). The area within the quadrant is shown under high power magnification in figure 5. Methylene blue and eosin; × 150.

of the just described minute "granulomas." Thus, here, too, there occurred an increase in the number of the cells alongside the wall of the air sac, and cells lying free in the respiratory vesicle could also be seen scattered here and there.

Whereas the aspect of the smaller cells was that of the lymphomonocytoid variety (and these cells are apparently the small nucleated cells of the respiratory alveolus), that of the larger ones, which are in all likelihood their direct descendants, was that of fixed or of wandering macrophages.

The lymphoid tissue showed nothing worthy of note.

A thorough search failed to reveal the existence of a so-called "membrana epithelia," for the cells that displayed the described activities "lined" the septums and therefore directly faced the lumen of the air sac.

After Five Minutes.—When the rabbit's lung was examined five minutes after the intratracheal injection, its gross appearance was likewise not remarkable. The

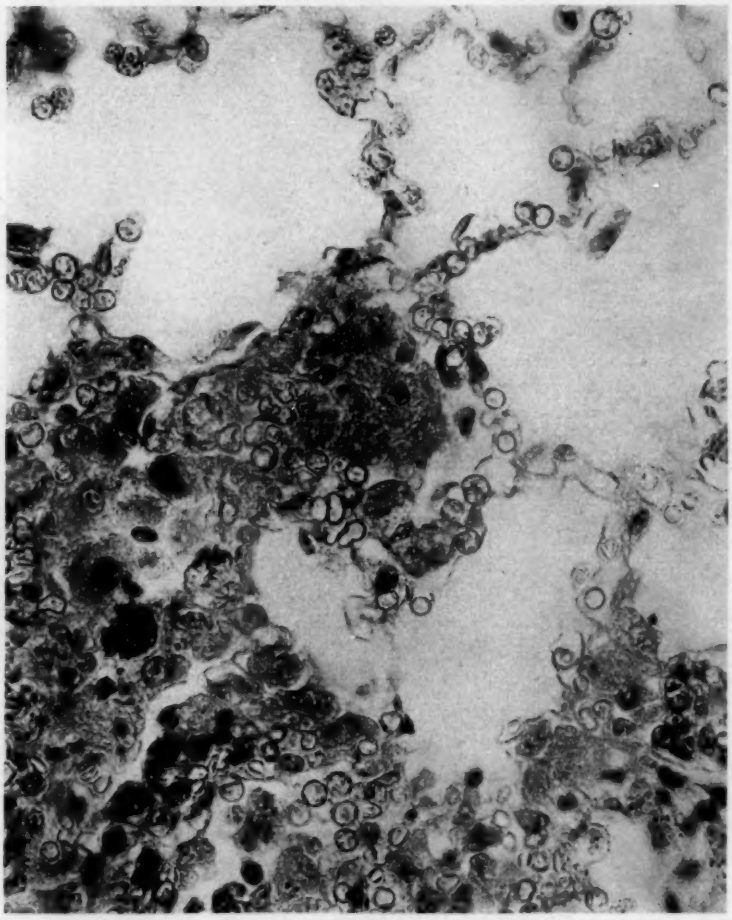

Fig. 5.—One detail of the "parenchymatous alveolitis" demonstrated in the previous figure, as seen under lenses of high power. The cells lining the walls of the alveoli, and not others, have given birth to the intra-alveolar "epithelioid" cells. The reaction is "proliferative" because of the fact that a proliferation of the "epithelial" cells has occurred. The cells adjacent to the "tubercle" should be noticed. Methylene blue and eosin; × 600.

histologic picture which is shown in figures 4 and 5, was as follows: The focal cellular agglomerations noticed immediately after the infection had increased in number and in size. They occupied several alveoli, the confines of which could still be made out. The cells filled the entire lumen of each air sac almost to

capacity. The cells were mostly of the large variety described; their nuclei were irregularly round, with coarse chromatin particles, and the cytoplasm was foamy with "ragged" outlines, containing, in some instances, tubercle bacilli. The agglomeration of the cells, which had all the marks of epithelioid cells, gave to these foci the appearance of "primitive tubercles."

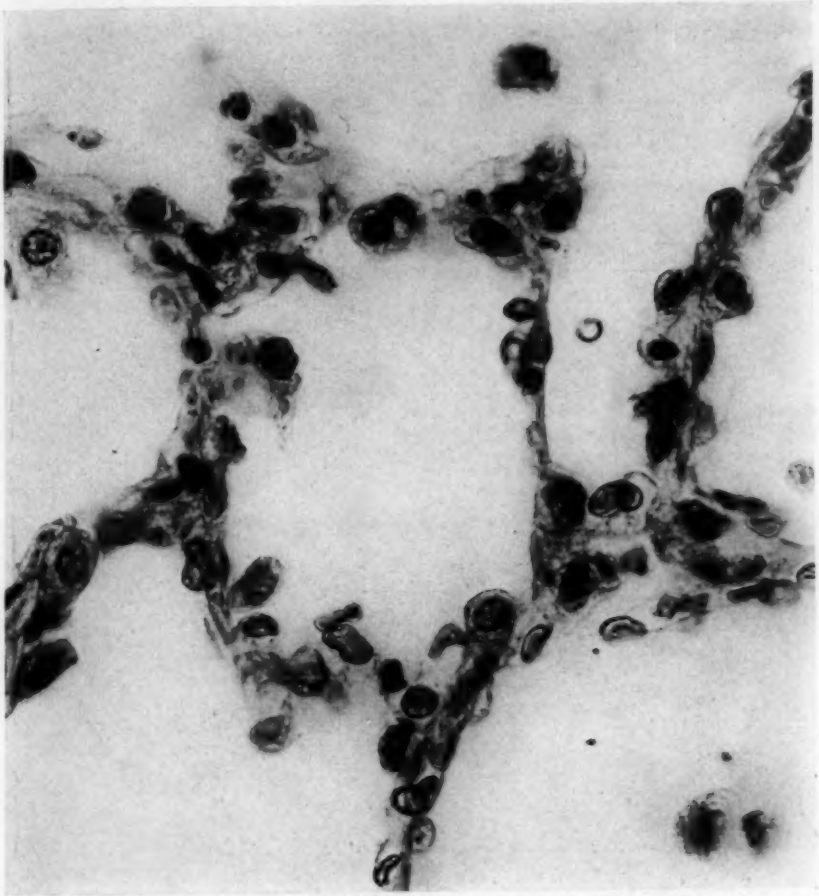

Fig. 6.—A section from an air sac near a tubercle found in the lung of an animal that died ninety hours after intratracheal infection. Note the topography and the morphology of the "resting" and of the "wandering" cells. Methylene blue and eosin; × 850.

Here, too, a thorough investigation of the tissue revealed that the endothelium of the septal capillaries remained passive; their lumina were but slightly filled with red cells.

It was obvious that the intra-alveolar exudate resulted from the cells lining the wall of the air sac, which had instantly undergone the changes just recorded. This, moreover, could be ascertained from the inspection of the sections at the border of the "primitive tubercle," which revealed the following: The tubercle

was rather sharply circumscribed. With the lenses of lower power one noticed merely an increase in the number of "nuclei" per microscopic field. But with the oil immersion lenses the topography and also the morphology of these cells could clearly be made out (fig. 6). They lay, as already said, outside on the wall of the respiratory vesicle facing the alveolar lumen. They were polymorphic, varying from a somewhat elongated endothelial-like form to a monocytoid variety; they occurred singly or in groups of two or three, and they showed morphologic transitions, as did the cells seen about one minute after the infection. They were more conspicuous at the intersection of the capillaries.

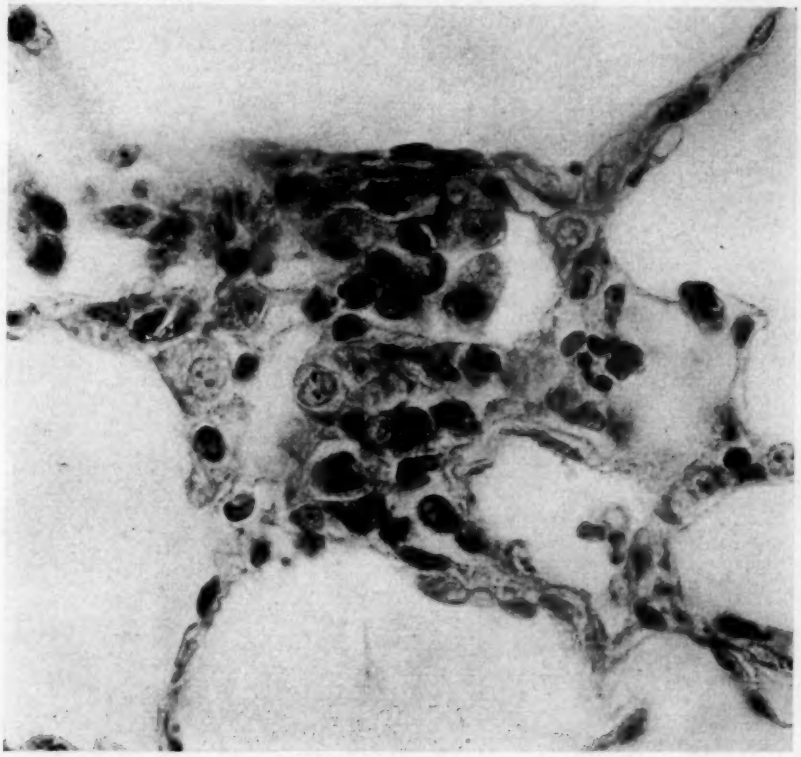

Fig. 7.—A "primitive tubercle" found in a rabbit's lung ten minutes after the intratracheal injection of bovine tubercle bacilli. Here, too, there occurred a "parenchymatous alveolitis" resulting from the proliferated "alveolar epithelial" cells. Methylene blue and eosin; × 850.

At this period one noticed more granulocytes and also a few pseudo-eosinophilic cells. There also were conspicuous proliferations of cells around the thin-walled veins.

There was no serum or blood in the alveoli, and the lymph follicles maintained their passive attitude. Here, too, no traces of a continuous "epithelial membrane" could be found.

After Ten Minutes.—A section from a lung removed ten minutes after the intratracheal infection is seen in figure 7. From this photomicrograph again it will be seen that the "tubercle" is intra-alveolar (parenchymatous alveolitis);

that it is made up of large epithelioid cells, and that the cells forming the tubercle and those lying close to the septum are identical as to the finest details.

After One Week.—The reaction increased in intensity every few minutes, the difference between the earliest response of the pulmonary tissues and that in the later periods being no more than quantitative.

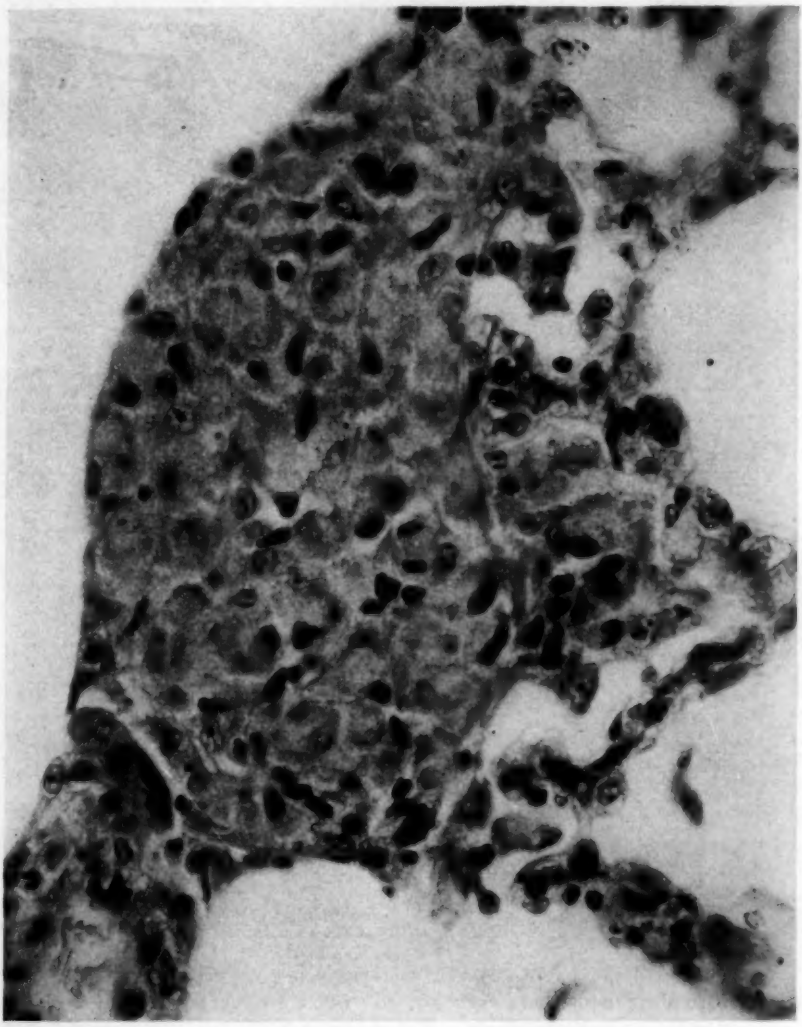

Fig. 8.—A "metastatic" miliary tubercle found in the lung of a rabbit that had been infected seven days previously. A cell in mitosis is seen in the center of the figure. Methylene blue and eosin; × 600.

With the spread of the disease, primitive tubercles united to form large areas of tuberculous granulation tissue, the centers of which were undergoing caseation. This, under the conditions of the present experiments, occurred at the fourth or

at the fifth day after infection. But even at this stage, the histogenesis of the primitive tubercle could be traced with reasonable certainty, provided the edge of a "granuloma" was examined, or if the investigation was directed toward areas where new "metastatic" tubercles were formed as a result of the dissemination of the acid-fast bacilli throughout the lung.

As an illustration, the structure of the tuberculous tissue seven days after the intratracheal injection of Koch's bacillus may be studied. This tissue is represented in figures 8, 9 and 10.

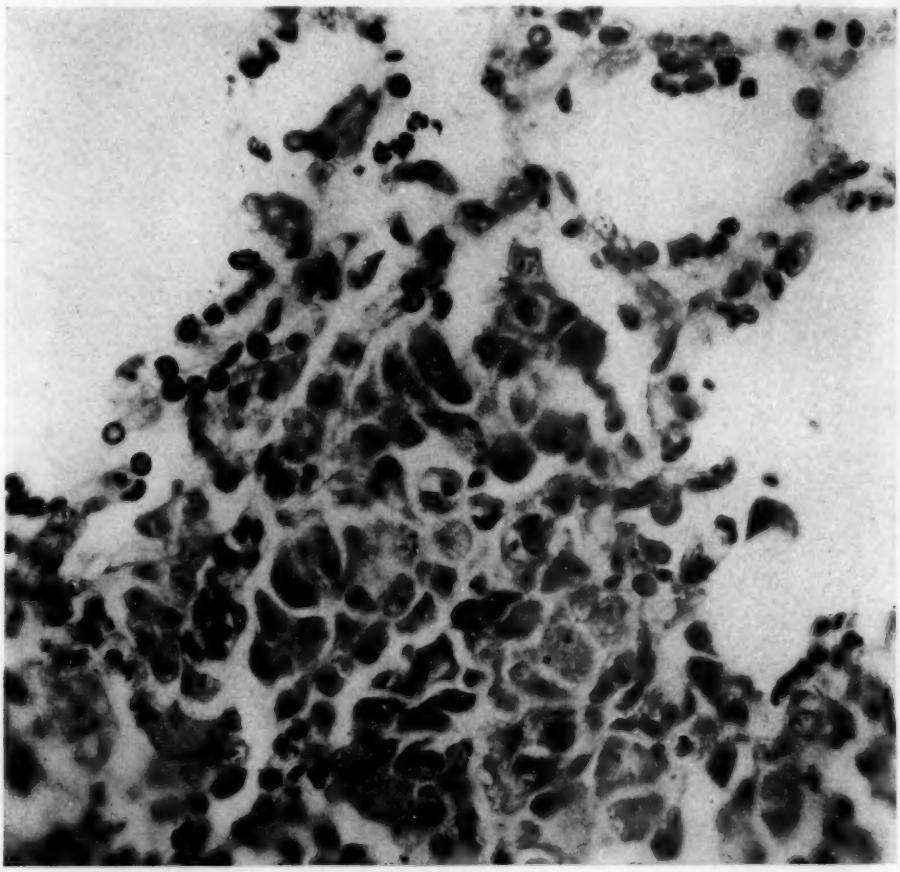

Fig. 9.—A 7 day old intra-alveolar tubercle. Note the cells lining the septum and compare them with those forming the "exudate." A few granulocytes are seen here and there. Methylene blue and eosin; × 600.

The tubercles illustrated in figures 8 and 9 were found in the lungs of two different animals. In the aerogenous infection 1 week old, the "metastatic" tubercle was a counterpart of what one had observed in the lungs five minutes after infection. The "alveolar epithelial" cells, and no others, evolved into typical epithelioid cells, which agglomerated to form the miliary tubercle. The morphology of the "epithelioid" cells is particularly demonstrated in figure 10. This

section, too, was made from the lung of a rabbit killed seven days after the intratracheal infection. The last three photomicrographs show, then, tuberculous lesions of the same age, yet these lesions reveal striking differences in their appearance, which indeed is characteristic of the "polyblastic" nature of the "alveolar epithelium."

There still exists a discussion among phthisiologists as to whether the primitive tubercle originates in the lumen of the alveolus or is formed within the connective

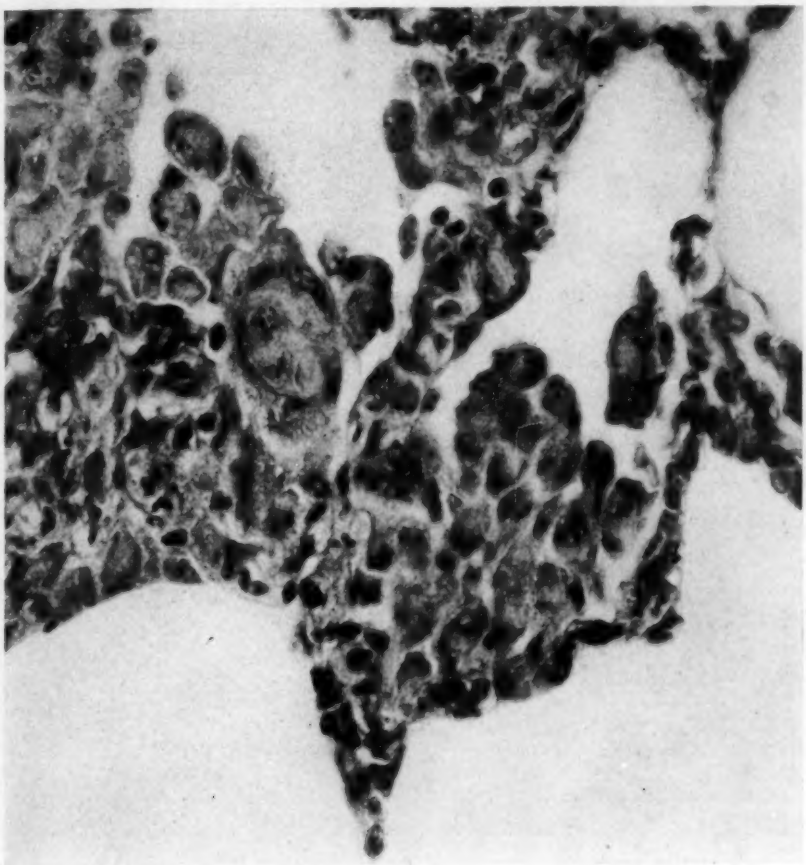

Fig. 10.—The histologic appearance of the tubercular infection of seven days' duration. The exquisitely typical "epithelioid" cells give the impression that they are "streaming" from the septum toward the infected alveolar lumen. Hematoxylin and eosin; × 600.

tissue of the septum. The experiments presented here have shown that the primitive tubercle is essentially an intra-alveolar structure, or what I should designate as a "parenchymatous alveolitis" (figs. 4, 7, 8 and 9). If one is to use the terminology of some German writers, the reaction is a proliferative one, because of the fact that there occurs a proliferation of the "alveolar epithelial" cells.

After Thirteen Days.—The border of the "granuloma" was of interest in still another way: The left segment in figure 11 shows a lesion of thirteen days' duration. From this picture it will be seen that the necrotic (caseated) center is surrounded by a rim of healthy cells. It is interesting that, at the border, the conglomerate tubercle was formed not only as a result of a proliferation of its own elements, but also through "conversion" of those "resting" on the alveolar wall.

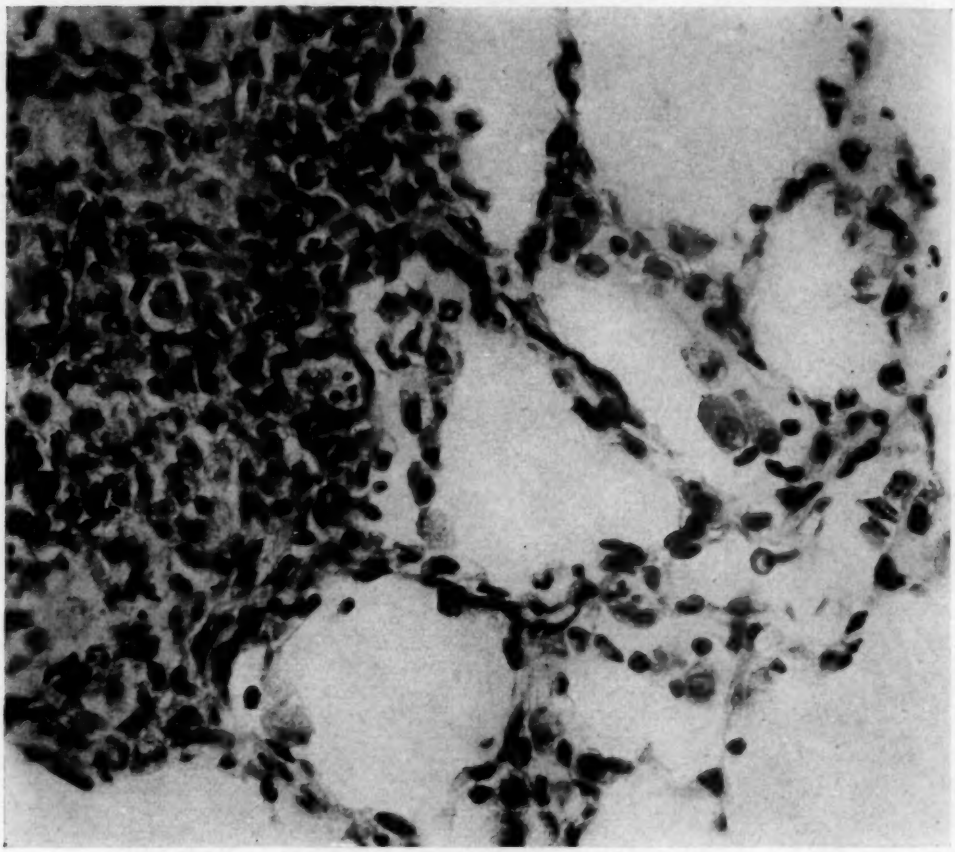

Fig. 11.—The picture as seen at the border of a tubercular granuloma of thirteen days' duration. The "conversion" of the adjacent "resting" cells and their "streaming" toward the aging focus are remarkable. Hematoxylin and eosin; × 600.

The formation of giant cells occurred within a few hours after the infection by way of fusion of "alveolar epithelial" cells and also by nuclear division of these cells.

In brief, then, at all stages of the tuberculous infection the essential response to the acid-fast bacilli in the respiratory alveolus was that of the cells "lining" the wall of the air sac, commonly designated as "respiratory epithelium."

When tubercle bacilli reached the peribronchial lymphoid tissue, they inaugurated a tubercle in the "clear zone" of the reticular cells, which evolved into

epithelioid cells. As a result of the proliferation of these cells the wide zone of the small round cells became thinner. However, a transformation of the lymphoid cells into macrophages and epithelioid cells could not be observed.

The perivascular and peribronchial mesenchymal cells, too, underwent morphologic changes when tubercle bacilli settled adjacent to them. They, moreover, proliferated, forming perivascular collars of macrophages. Perivascular agglomerations of thick layers of small round cells could not be seen in the lungs of the tuberculous rabbits, like those found in the "model infections" of rabbits or of cats with oils [4] (fig. 12).

In the bronchi progressive changes were seen in the so-called basal cells of the bronchial mucosa, evidenced by focal proliferation of the cells and also by occa-

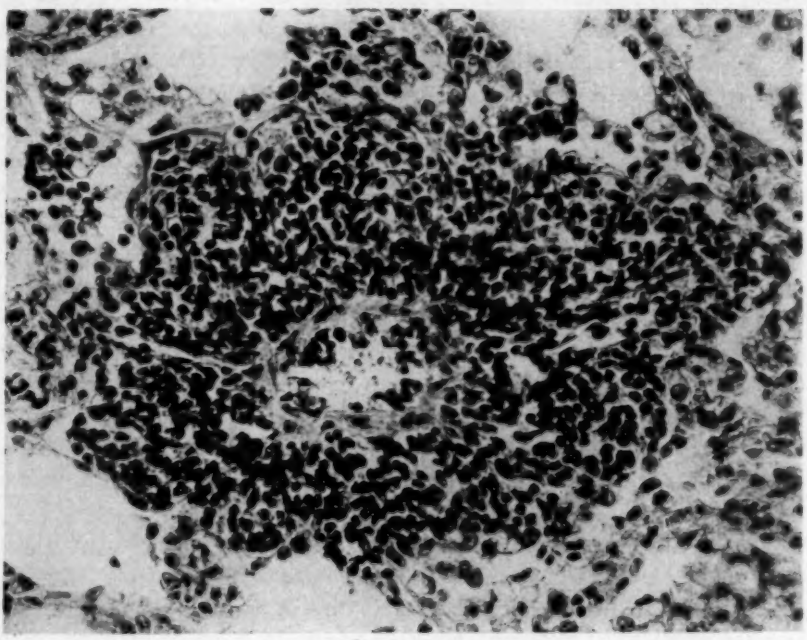

Fig. 12.—Perivascular proliferation of lymphoid cells in the lung of a cat that had received one injection of oil by way of the trachea. Hematoxylin and eosin; × 300.

sional mitoses. The columnar cells revealed early regressive changes ending in complete degeneration near tuberculous granulation tissue. The notion of pathologists of the past century (recently revived by Duthie [5]) that the bronchial epithelium is actively phagocytic for bacteria, being a source of the "dust cells," was not borne out by these experiments. I have observed that the alveolar wandering macrophages ("dust" cells) often "invade" the bronchial lumen and wall as well, this certainly leading to the erroneous impression of the transformation of the columnar bronchial epithelium into "dust" cells.

4. Fried (footnote 1, second reference).

5. Duthie, E. S.: Phagocytosis by Bronchial Epithelium in the Lungs of Mice, J. Path. & Bact. **33**:547, 1930.

COMMENT

The histogenesis of the tubercle in the lung is as debatable today as it was decades ago. One is still uncertain not only of the strain, but also of the source of the cells of which the primitive pulmonary tubercle is made up.

The discussion has become particularly acute since the inauguration of the teachings of Metchnikoff on inflammation. Baumgarten,[6] who was the first to study experimental tuberculosis with the then discovered tubercle bacillus is often quoted as being the original author of the theory whereby the fixed tissue cells were said to be responsible for the tubercle, while Metchnikoff [7] is referred to as the partisan of the hematogenous origin of the pulmonary epithelioid cell. In reality the "fixed cell" theory was the one that prevailed among workers of the past.

The conception of Virchow that the tuberculous granulation tissue develops only from the connective tissue did not receive general recognition by his contemporaries. Reports by numerous workers were to the effect that every cell in the organism took part in the formation of the tubercle. A French writer of the seventies of the past century, who gave a good summary of the question, wrote: "The tuberculous granulation tissue has the sad privilege to be developed at the expense of the cells of the connective tissue, of the epithelium of the serous membrane in the lungs, of the hepatic cells, of the renal cells, and of the smooth muscle fibers" (Thaon,[8] Grancher [9]). In Germany similar views were simultaneously expressed in a detailed study on tuberculosis by Arnold.[10]

The results obtained by Baumgarten [6] from his experimental studies with Koch's bacillus did not differ from those of his predecessors. He, too, traced the origin of the tubercle to the endothelial cells, to the hepatic and biliary epithelial cells, to the renal epithelium and also to the bronchial and the respiratory (alveolar) epithelium.

The early investigations by Metchnikoff [11] revealed, however, that the phagocytosis of the tubercle bacillus is the essential function of

6. Baumgarten, P.: Experimentelle und pathologisch-anatomische Untersuchungen über Tuberkulose, Ztschr. f. klin. Med. **9**:93 and 243, 1885; **10**:24, 1886.

7. Metchnikoff, E.: Leçons sur la pathologie comparée de l'inflammation, Paris, Masson & Cie, 1892.

8. Thaon, L.: Recherches sur l'anatomie pathologique de la tuberculose, Paris, Duval, 1873.

9. Grancher, J.: De l'unité de la phtisie, Thèse de Paris, 1873; Tuberculose pulmonaire, Arch. de physiol. norm. et path. **10**:2, 1878.

10. Arnold, Julius: Beiträge zur Anatomie des miliären Tuberkels: IV. Ueber disseminierte Miliartuberkulose der Lungen, Virchows Arch. f. path. Anat. **88**: 397, 1882.

11. Metchnikoff, E.: Ueber die phagocytäre Rolle die Tuberkelriesenzellen, Virchows Arch. f. path. Anat. **113**:63, 1888; footnote 7.

the macrophage. He therefore emphasized the fact that: "le tubercle est composé d'une réunion de phagocytes d'origine mésodermique qui affluent vers les endroits ou se trouvent les bacilles et les englobent." ("The tubercle is made up of a gathering of phagocytes of mesodermic origin, which flock toward the areas where the bacilli are located and engulf them.")

Although this conception gained ground, it was still believed that in pathologic conditions of the lungs the alveolar epithelium was the essential active element. An investigation by Arnold [12] on pneumonoconiosis showed to him that the earliest and most constant effect of the inhaled dust was conspicuous in the alveolar epithelium. Ribbert (cf. Fleck [13] and Laehr [14]), by infecting animals with streptococci and staphylococci, respectively, noted that the micro-organisms were actively phagocytosed by the respiratory epithelium. (This served as an argument against the conception that active phagocytosis is the monopoly of the "so genannte Makrophagen" [Ribbert]).

Studies on pneumonia in man have likewise revealed the readiness with which the "alveolar epithelial" cells respond in acute inflammatory conditions of the lungs. Of particular interest were the observations made on the so-called "pneumonia desquamativa" which was for the first time reported by Buhl.[15] The disease was said to begin with morphologic changes in the alveolar epithelium, with proliferation of these cells, and then "desquamation," forming the intra-alveolar exudate. In the obliterating forms of this disease (pneumonia desquamativa obliterans) the reticulum, too, was claimed to be formed by the respiratory epithelium (Galdi [16]). Cornil,[17] who, with many others, regarded the endothelium of blood and lymph channels as being the essential element in the field of inflammation, compared the function of the respiratory epithelium to that of the lining of the blood vessels. He wrote, for instance "The phenomenon of beginning inflammation is essentially the same whether it concerns the connective tissue or the lungs."

Apparently the "endothelial" theory of inflammation began to prevail, for in his lectures on inflammation Metchnikoff [7] stated that "the cells

12. Arnold, Julius: Staubinhalation und Staubmetastase, Leipzig, F. C. W. Vogel, 1885.

13. Fleck: Die acute Entzündungen der Lunge, Dissertation, Bonn, 1886.

14. Laehr, G.: Ueber den Untergang des Staphylococcus pyogenes aureus in den durch ihn herforgerufenen Entzündungsprozessen, Dissertation, Bonn, 1887.

15. Buhl, L.: Lungenentzündung, Tuberkulose und Schwindsucht. Zwölf Briefe an einen Freund, ed. 2, Munich, R. Oldenbourg, 1873.

16. Galdi, F.: Ueber einige von den gewöhnlichen abweichende Pneumonie-Formen, Deutsches Arch. f. klin. Med. **75**:239, 1903.

17. Cornil, V.: De modifications que subissent les cellules endotheliales dans les inflammations, Arch. de méd. expér. et d'anat. path. **9**:9, 1897.

in tuberculosis (the epithelioid as well as the giant cells) are made up exclusively of phagocytic elements; that is, of the large mononuclear leukocytes and of the stellate Kupffer cells which are of endothelial origin; in the spleen and the lymph nodes the tubercle results from a collection of the large mononuclear phagocytes *of these organs,* and in the lungs it is formed at the expense of the endothelial cells of the blood vessels with the assistance of the leukocytes." Metchnikoff did not mention the origin of the pulmonary "leukocytes" until a few years later when he [18] wrote: "For long the large dust cells of the respiratory channels were looked on as being epithelial cells that were capable of taking up carbon particles, micro-organisms and other foreign bodies. In reality these elements are nothing more than white corpuscles that have immigrated into the alveoli and bronchi."

The apparent inconsistency of Metchnikoff in regard to the inflammatory reactions in tuberculosis of the lungs undoubtedly resulted from the fact that the conception of the fixed macrophages (histiocytes) was not clearly understood by pathologists of that period. The clasmatocyte described by Ranvier [19] was thought to be of hematogenous origin, being present in the taches laiteuses of the omentum only, and their virtual significance as well as their relation to the histiocytic elements found elsewhere in the body was not appreciated.

More recent studies on mesenchymal reactions in tuberculosis of organs other than the lungs have shown that the primary reaction to Koch's bacillus is essentially that of the local elements (Buday;[20] Joest and Emshoff;[21] Evans, Winternitz and Bowman;[22] Krause [23]). The question of the source of the "exudate" in inflammatory and congestive conditions of the lungs, and particularly of the cells that form the pulmonary tubercle, has remained controversial.

The experiments presented here have shown, first, that in tuberculosis of the lung, too, the cells that primarily respond to Koch's bacillus are not called from outside sources, but are produced in situ. In the second place, they have shown that the source of these cells is not the

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18. Metchnikoff, E.: L'immunité dans les maladies infectieuses, Paris, Masson & Cie, 1901.

19. Ranvier, L.: Des clasmatocytes, Arch. d'anat. micr. **3**:123, 1900.

20. Buday, K.: Experimentelle histologische Studien über die Genese der Miliartuberkels, Virchows Arch. f. path. Anat. **186**:145, 1906.

21. Joest, E., and Emshoff, E.: Studien über die histogenese des Lymphdrüsentuberkels und die Frühstadien der Lymphdrüsentuberkulose, Virchows Arch. f. path. Anat. **210**:188, 1912.

22. Evans; Winternitz, and Bowman: An Experimental Study of the Histogenesis of the Miliary Tubercle in Vitally Stained Rabbits, J. Exper. Med. **19**:15, 1914.

23. Krause, A. K.: The Anatomical Structure of the Tubercle from Histogenesis to Cavity, Am. Rev. Tuberc. **4**:137, 1927.

endothelium of the pulmonary capillaries (endothelial leukocytes), but cells that "line" the walls of the air sacs, as well as those that are normally present in the loose connective tissue of the septums.

Let me analyze my observations: When the tubercle bacilli reached the air sac, the reaction caused by them was instantaneous. For within one minute after the aerogenous infection (figs. 1, 2 and 3), there occurred a marked proliferation, as well as morphologic changes, of cells lying in and on the septums. These cells, which are normally barely visible, giving to the air sac a "naked" appearance, revealed characteristics that are proper to fixed or to wandering macrophages (monocytes and clasmatocytes). A number of these cells lay free in the alveolar lumen, showing phagocytosis of the tubercle bacilli; the bacilli also were found in the cytoplasm of some of the enlarged sessile cells. Cells in mitosis and in amitosis were seen here and there, and within the next few minutes primitive tubercles made up of these (epithelial) cells transformed into epithelioid cells were seen scattered over the sections. At this period, too, outlines of giant cells could be made out budding from the septums, resulting from the "agglutination" of individual cells and also from the nuclear division of cells.

As a rule the newly formed cells proliferated inside the lumen of the alveolus, causing a "parenchymatous alveolitis." This process ordinarily involved a group of several alveoli, of which the smaller were completely occluded by the epithelioid cells, while the large were occupied only in part.

It is remarkable, then, that in addition to the prompt morphologic changes and the rapid proliferation, the sessile and the free cells also displayed an instantaneous phagocytosis of the acid-fast bacilli.

The endothelium of the capillaries of the septums showed no particular changes. Their lumina contained a moderate number of red cells, but no mononuclear leukocytic elements.

Particularly instructive, too, were the pictures in close proximity to the primitive tubercle. It appeared that not only did the morbid process advance by proliferation of its own elements, but at the borders it also "converted" the adjacent cells. Here, also, the cells while still "resting" on the septums, had taken on new morphologic aspects, thus giving them the appearance of large monocytes (fig. 13). In proximity to the primitive tubercle these cells could be seen to "stream" singly or in groups to join and to enforce the already formed "miliary granule." Thus, when the agglomerated tubercle had reached considerable dimensions, and its center had begun to degenerate (caseation), its periphery was made up of new elements that resulted not only from proliferation of its own cells, but also from the mobilization of those resting in the adjacent areas (figs. 11 and 13).

As I have said, the reaction was confined to the cells lining the septums and to those found within the septums as well. The wall of the air sac, representing, indeed, no more than a "spider's webb" of loose connective tissue enforced with a few elastic fibers, also contains interlacing bundles of innumerable capillaries and a sparse number of scattered cells. These cells, when sectioned at various angles, appear either as elongated cells lying "astride" on the capillary, as do the

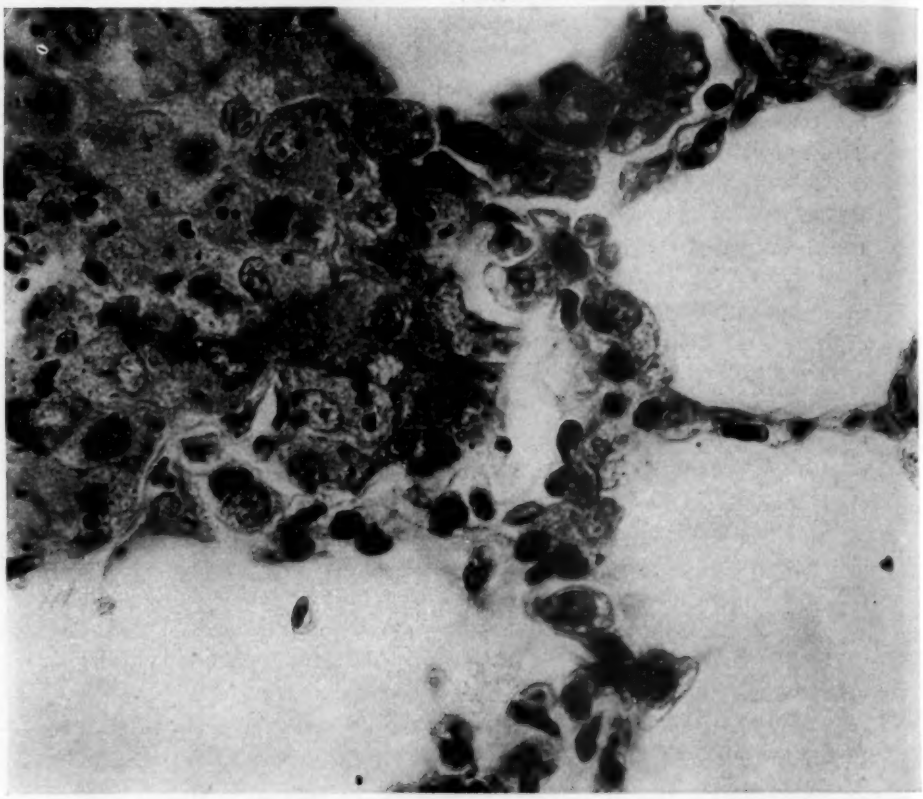

Fig. 13.—Note the cells "lining" the walls of the air sacs at the frontier of an advancing tubercle. Compare these cells with those that had formed the "miliary granule" seen on the left side of the picture. Methylene blue and eosin; × 800.

"adventitial" cells of Marchand, or as round lymphocytoid cells having somewhat bean-shaped nuclei with coarse, darkly stained chromatin particles. The resemblance of these cells to lymphocytes is remarkable, and was probably responsible for the opinion expressed by Metchnikoff that the lymphocytes are to a large degree the source of the epithelioid cells. "Si les lymphocytes même," wrote Metchnikoff,[7] "ne sont encore point des phagocytes, ils le deviennent bientôt, après s'être transformé

en cellules epithelioides." ("If the lymphocytes," wrote Metchnikoff, "are not yet phagocytes, they soon become so after having been transformed into epithelioid cells.") This property of the small round cell was also stressed by Maximow,[24] who was, however, of the opinion that "the small lymphocytes, as a rule, first have to enter the blood and to circulate there before being activated for the progressive development under the influence of suitable external conditions." Bloom [25] produced convincing experimental evidences of such a metamorphosis in vitro, but Ferrata [26] and Naegeli [27] were opposed to this view.

In the experiments presented here, the pulmonary lymphocytes did not seem to play a rôle in the provision of macrophages. For even in the peribronchial lymphoid accumulations the tubercle developed in the midst of the "clear zone" of reticular cells, which gradually had evolved into epithelioid cells, but no transitions could be seen between these cells and the small round cells. Likewise the fully developed tubercle in these cases was composed of a central giant cell surrounded by epithelioid cells with a very scant number of "small round cells," unlike the picture one ordinarily sees in tubercles found in protracted cases of human tuberculosis.

Apparently the cells found in the walls of the septums are not other than undifferentiated mesenchymal cells, which here, as indeed elsewhere in the organism, readily serve as a source of the wandering macrophage.

The topography of these small lymphocytoid cells about the capillaries of the air sacs imitates the same condition found throughout the body, and the suggestion was made in a recent textbook of histology [28] that they are probably nothing other than adventitial cells.

That the adventitia of blood channels is accompanied by peculiar cells was for the first time demonstrated by Rouget,[29] who believed them to be "muscle cells" (cellules de Rouget) playing a rôle in the

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24. Maximow, A.: Handbuch der microscopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 1, p. 2.

25. Bloom, W.: Ueber die Verwandlung der Lymphozyten der Lymphe der Ductus thoracicus der Kaninchens in Polyblasten (Makrophagen) in Gewebskulturen, Centralbl. f. allg. Path. u. path. Anat. **39**:3, 1927.

26. Ferrata, A., quoted by Maximow, A.: The Lymphocytes and Plasma Cells, Special Cytology, edited by E. V. Cowdry, New York, Paul B. Hoeber, 1928, vol. 1, p. 321.

27. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, ed. 4, Berlin, Julius Springer, 1923.

28. Maximow, A.: A Text-Book of Histology, Philadelphia, W. B. Saunders Company, 1931.

29. Rouget, C.: Mémoire sur le development, la structure et les propriétés physiologiques des capillaries sanguins et lymphatiques, Arch. de physiol. norm. et path. **5**:603 and 639, 1873.

contraction of the capillaries. Eberth [30] observed "perithelial" cells around the blood vessels of the central nervous system, which he thought were epithelial. ("Peritheliomas" were believed to originate from these cells.) Marchand [31] found them to be an essential part of the vascular adventitia (adventitial cells of Marchand). Goldmann [32] convincingly demonstrated that these cells belong to the reticulo-endothelial (macrophage) system. Krogh [33] and his pupil Vimtrup [34] confirmed the observations of their predecessors, and a Swiss histologist, Zimmermann,[35] designated them as pericytes (figs. 14 and 15).

Whatever the nomenclature of these cells may be, they are, it is reasonably certain, of mesenchymal origin, and act as such: they store dyes and oils; they avidly phagocytose micro-organisms; they play a

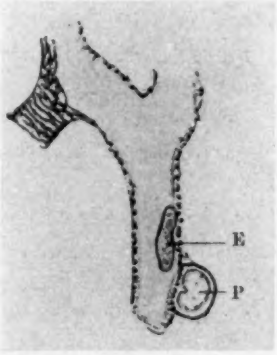

Fig. 14.—A capillary from the thymus: *E*, endothelium; *P*, pericyte. (After Plenk; copied by Benninghoff, A.: Blutefässe und Herz, in Möllendoff: Handbuch der microscopischen Anatomie des Menschen, Berlin, Julius Springer, 1925, vol. 6, p. 30).

rôle in the extrahepatic formation of bile; [36] they likewise participate in the formation of the tubercle, as seen in the experiments presented here.

30. Eberth, C. J.: Zur Entwicklung des Epitheliomas (Cholesteatomas) der Pia und der Meningen, Virchows Arch. f. path. Anat. **49**:48, 1870.

31. Marchand, F., in Krehl and Marchand: Handbuch der allgemeinen Pathologie, Leipzig, S. Hirzel, 1924, vol. 4.

32. Goldmann, E. A.: Die äussere und innere Secretion des gesunden und kranken Organismus im Lichte der "vitalen Färbung", Beitr. z. klin. Chir. **64**:192, 1900; **78**:1, 1912.

33. Krogh, A.: Anatomy and Physiology of Capillaries, New Haven, Conn., Yale University Press, 1928.

34. Vimtrup, B.: Beitrag zur Anatomie der Capillären, Ztschr. f. Anat. u. Entwcklngsgesch. **65**:150, 1922.

35. Zimmermann, K.: Der feinere Bau der Blutcapillären, Ztschr. f. Anat. u. Entwcklngsgesch. **68**:29, 1923.

36. Fried, B. M.: The Rôle of the "Alveolar Epithelium" in the Extrahepatic Formation of Bile, unpublished data.

The "defensive" rôle of the lung is obvious not only from the instantaneous response of its local (alveolar wall) macrophages, but from the natural history of the aerogenous infections with Koch's bacillus. Thus, under the conditions of the experiments discussed in this article, rabbits that have been infected with the bovine type of tubercle bacilli by way of the trachea survive three and more times

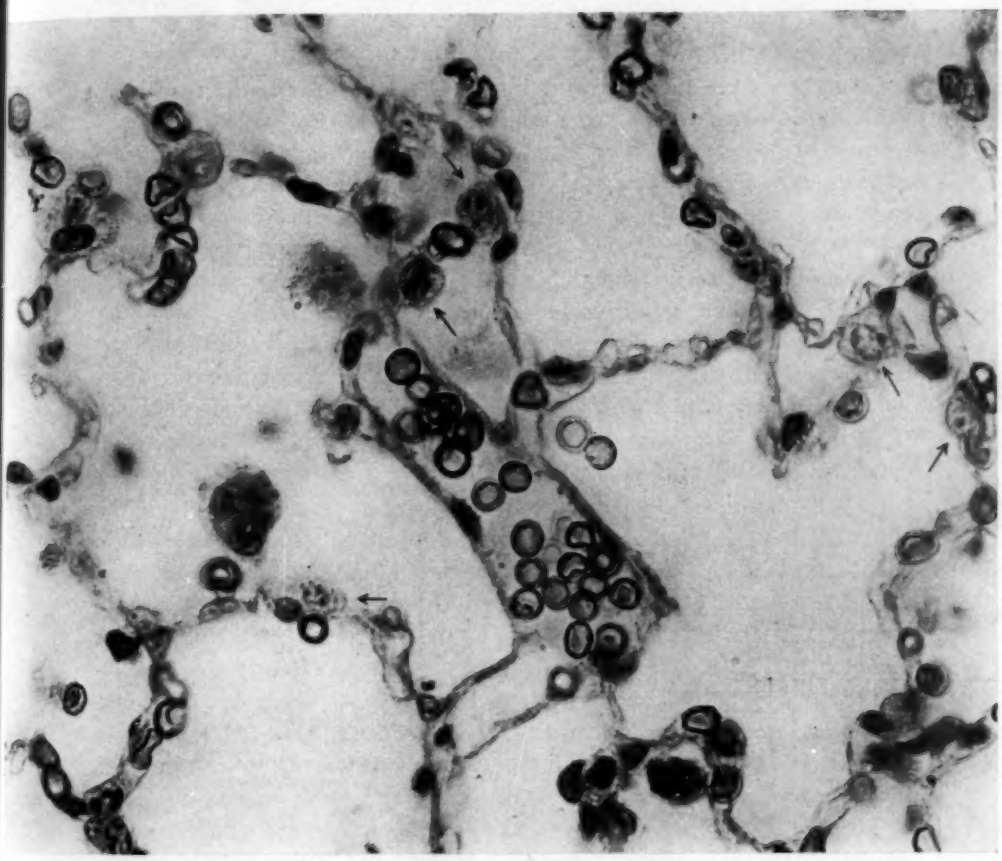

Fig. 15.—An area of a rabbit's lung remote from tuberculous granulation tissue. The cells around the capillaries should be noticed. Compare their topography with that of the pericyte (*P*) shown in figure 14. Methylene blue and eosin; × 850.

longer than those that have received a similar dose of bacilli by way of the blood stream. It also is interesting that in many animals that have been infected via the trachea the acid-fast micro-organisms apparently remain "immured" in the lung "for life," for in these instances the micro-organisms cannot be detected outside these organs. The lungs,

then, are not only a "sieve" or a "filter" (Spanier [37]), but are a virtual "fortress" which retains and destroys not only foreign elements, but pathogenic micro-organisms (staphylococci, anthrax bacilli, pneumococci).

It is true that hematogenous elements (monocytes and also lymphocytes) contribute to the formation of the tuberculous granulation tissue of the lung. However, the afflux of these cells from outside sources occurs at a later date and obviously does not play an important rôle when infection occurs by way of the respiratory tract.

It is of interest that even granulocytes, which are found in numbers in the lungs of rabbits infected with tubercle bacilli by way of the blood stream, are insignificant in aerogenous infections. The rapid appearance of these cells in the lungs after infection was thought by Töppich [38] to be the result of their local formation from the "younger mesenchymal cells." In reality the granulocytes reach the lung from the periphery.

Studies on the peripheral blood of animals infected with tubercle bacilli [39] reveal that the infection is followed by an almost instantaneous leukopenia. The granulocytes, then, that have disappeared from the circulation, are found in the visceral organs and particularly in the lungs. They often contain engulfed tubercle bacilli, and in their turn are phagocytosed by the local macrophages. (This will be discussed in detail in a report on hematogenous infection with tubercle bacilli.)

That the lungs react as do mesenchymal organs, and that the essential source of the reactive elements is not the blood or the endothelium of the capillaries, but the cells lining the air sacs, as well as those found within the septums, is apparently not contested any more. What one disputes today is the "alveolar epithelium," the mesenchymal nature of which is denied on the ground that it is in conflict with the "classic" descriptions of the embryology of the lung. However, recent investigations of the fetal lung revealed that there exists a great uncertainty as to the embryology of the primordium of the air sacs. Thus Rose [40] produced evidences to show that the lung is developed from two different germ layers, endoderm for the bronchi and mesoderm for the "parenchyma." Bloom, in Maximow's [28] textbook of histology, was as yet undecided, urging "a thorough embryological

37. Spanier, Philipp: Ueber die Bedeutung der Lungen im Stofftransport innerhalb der Organismus, Eine experimentelle Untersuchung, Beitr. z. Klin. d. Tuberk. **76**:507, 1931.

38. Töppich, G.: Die Zellulären Abwehrvorgänge in der Lunge bei Erst und Wiederinfektion mit Tuberkelbazillen, Krankheitsforschung **9**:15, 1925.

39. Fried, B. M., and Dameshek, William: The Picture of the Peripheral Blood in Rabbits Infected with the Tubercle Bacillus by Way of the Trachea, unpublished data; Experimental Agranulocytosis, Arch. Int. Med., to be published.

40. Rose, S. B.: The Finer Structure of the Lung, Arch. Path. **6**:36, 1928.

investigation of the lungs in the later stages of intra-uterine life," while Policard [41] and Chiodi [42] believed that originally there is a cuboidal epithelium lining the air sacs, but that it disappears in the later months of fetal life to be replaced by mesenchymal cells.

In fact the histologists' description to the effect that in the adult lung there is a transitional epithelium between the bronchiolic epithelium and that of the supposed cuboidal alveolar epithelium was not supported by

Fig. 16.—A segment of a respiratory bronchiole to show that there is no transitional epithelium between the bronchiolar epithelium and the alleged alveolar epithelium. The abrupt ending of columnar cells that line the bronchiole is indicated by an arrow. Methylene blue and eosin; × 600.

41. Policard, A.: Les nouvelles idées sur la disposition de la surface respiratoire, Presse méd. **37**:1243, 1929.

42. Chiodi, V.: Sulla natura delle cellule libere del pulmone e del rivestimento dell'alveolo pulmonare, Arch. d'anat., d'histol. et d'embryol. **8**:313, 1928.

these studies. From figure 16 it may be seen that the epithelium of the bronchiole ends abruptly, and is "followed" by the "naked" walls of the air sacs (fig. 16).

The assertion of the epithelial nature of the "alveolar epithelium" was also based on the observation that these elements, which are incon-

Fig. 17.—The cuboidal cells lining the glandular structures were interpreted by older pathologists as alveolar epithelium that had "reclaimed" its fetal cuboidal aspect. In reality these are cells of bronchiolar origin. The same cells, single and the groups, can be seen scattered throughout the photomicrograph. (Patient studied at the Beth Israel Hospital, Boston). Hematoxylin and eosin; × 300.

spicuous in normal tissues, "reclaim" their ancestral features in pathologic conditions, taking a fetal cuboidal aspect. The so-called "alveolar

cell" cancers of the lung were traced to these structures.[43] In reality the "glandular structures" observed in sclerosed lungs are nothing other than "alveoli" lined by cells of bronchiolar origin (fig. 17). This condition observed in the sclerosed lung is a counterpart of what one sees in atrophic (Laënnec's) cirrhosis of the liver, in which there also occurs a new formation of bile ducts in the connective tissue encircling the hepatic lobules.

As mentioned, the view of the mesenchymal nature of the lining of the air sacs is at present shared by many pathologists. But even those who still maintain that the alveoli are lined by epithelial cells have come to accept my conception of the "defensive and metabolic" functions of the alveolar cells, which is indeed the essential. It is interesting that these writers regard the histiocytic potentialities of the "respiratory epithelium" as merely an exception in the bodily economy. In this respect, the opinion of Renan[44] may be quoted. In his inauguration discourse at the Academie Française, where he was elected to take the chair of Claude Bernard, Renan said: "Le mot *d'exception* est anti-scientifique. Ce qu'on appelle exception est un phénomène dont une ou plusieurs conditions sont inconnues." ("The word 'exception' is anti-scientific; what is called an exception is a phenomenon, one or more conditions of which are not known.")

SUMMARY

Infection of rabbits with the bovine type of tubercle bacilli by way of the trachea provokes an instantaneous reaction in the lungs.

The early lesion, that is, the "primitive tubercle," is found in the lung within five minutes after the aerogenous infection. The lesion is intra-alveolar, being a "parenchymatous alveolitis."

The cells that primarily respond to the acid-fast bacilli are those commonly designated as "respiratory epithelium." Under the influence of Koch's bacillus, these cells undergo instantaneous morphologic changes, proliferate, separate from the wall of the air sacs, phagocytose the acid-fast bacillus and form typical miliary tubercles. They also form giant cells of the Langhans type.

Rabbits infected with bovine tubercle bacilli by way of the trachea survive from three to four times longer than those infected with the same amount of bacilli by way of the blood stream. In many of the aerogenously infected animals the acid-fast bacilli remain apparently

43. Fried, B. M.: Primary Carcinoma of the Lungs; Bronchiogenic Cancer, Baltimore, Williams & Wilkins Company, to be published.

44. Renan, Ernst: L'Œuvre de Claude Bernard, Paris, Baillière, 1881.

"immured for life" in the lungs, for no lesions or bacilli are detected in organs outside the pulmonary tissues.

The pulmonary reaction to the "virtual" infection with the Koch's bacillus is akin to that of the "model infections" with vital dyes and oils reported previously.[1]

THE SITES OF DECALCIFICATION AND OF BONE LESIONS IN EXPERIMENTAL HYPERPARATHYROIDISM *

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Recent clinical and chemical studies have demonstrated the ease with which the mineral constituents of the bony skeleton are mobilized and depleted in a variety of conditions. Few discussions exist concerning the varying degrees of susceptibility to decalcification of the various portions of the bony skeleton or of individual bones. Nor are the relations between decalcification in various sites and the corresponding pathologic pictures much discussed. With these relations in mind, we examined histologically the carpal, tarsal, metacarpal, metatarsal, phalangeal and other bones (like the apophyses and sesamoids) of guinea-pigs with hyperparathyroidism, in addition to the bones previously investigated, such as the long tubular bones, ribs, skull and jaw. The distribution of lesions in the bones of young guinea-pigs suffering from experimental scurvy was also studied and compared with that in guinea-pigs showing hyperparathyroidism.

THE DIFFERENTIAL GROWTH OF BONE

Certain features in the embryogenesis and postnatal development of the skeleton have a distinct bearing on the interpretation of our pathologic observations and on the conclusions based on them. To some degree, the succession of events in the development of the skeleton of man and that in the ordinary laboratory animal run parallel. Strong [1] drew certain analogies between the order of ossification in the rat and that in man. It is well known that certain bones reach their final forms and complete maturity much sooner than others. While species differ-

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* Aided by a grant from the Herbert L. Celler Research Fund.

* The parathormone used in the experiments was supplied by Eli Lilly and Company.

1. Strong, R. M.: Am. J. Anat. **36**:313, 1925.

ences exist in this respect, the progress of ossification within a given species is constant.

Ossification begins in man in the clavicle when the embryo is 15 mm. long (crown-rump) and 39 days of age. Numerous centers of ossification for the formation of other bones appear rapidly thereafter, and at birth the diaphyses of all the tubular bones are formed. At the time of birth, there are no centers of ossification in the cartilaginous epiphyses of the long tubular bones, except in the lower end of each femur, where a center is generally present, and in the upper end of each tibia, where one is occasionally present.[2] The epiphyses are formed by endochondral ossification. This progresses after the centers of ossification appear; most of the cartilaginous mass is soon replaced by bone. Further growth of the epiphysis is slow after this, and takes place along the line of junction of the bone and the remaining cartilaginous portion of the epiphysis. Finally, when the bony epiphysis is completely formed, an irregular cement line marks the junction between the bone and the articular cartilage, along which there is slow progressive erosion of the cartilage and formation of new bone by creeping replacement. The spongy trabeculae of the epiphysis are composed of fine-fibered lamellar bone, and no haversian canals are present except in the larger ones. The reconstruction of these fully formed trabeculae also proceeds slowly.

The epiphyses of the short tubular bones (metacarpals, metatarsals and phalanges) ossify rapidly, reaching their complete maturity not long after puberty; the epiphyses and diaphyses of these bones fuse and the epiphyseal cartilage plates disappear. On the other hand, fusion of the epiphyses of the lower ends of the femurs, of the upper ends of the tibiae and of the upper ends of the humeri does not occur in man until at least the nineteenth or the twentieth year. Like the epiphyses, the tarsal and carpal bones, with the exception of the os calcis, are entirely formed by endochondral ossification. These bones ossify rapidly after the appearance of centers of ossification in them, and thereafter grow slowly at the periphery beneath the cartilage. The exception, the os calcis, has an epiphysis at its posterior extremity.

On the metaphyseal surfaces of all epiphyseal cartilage plates new bone is formed as long as the plates remain active, but bone formation is most abundant in these regions in bones of greatest growth. There is a progressive diminution leading finally to complete cessation of ossification, as the epiphyseal cartilage plates go through the stages of closing and disappearing. In man, closure of the epiphyseal cartilage plate is followed by its rapid disappearance, resulting in fusion of the epiphysis and diaphysis, but in certain animals the plate may close without disap-

2. Adair, F. L., and Scammon, R. E.: Am. J. Obst. & Gynec. **2**:35, 1921.

pearing for a long time. Dawson [3] showed that in the rat the epiphyseal cartilage plates of the upper ends of the humeri, of the lower ends of the femurs and of the upper ends of the tibiae remain until senility.

Bone formation proceeds very actively at the costochondral junctions until maturity. Furthermore, it may be noted that the jawbone is almost continually in a state of active growth and transformation. This is because it is subject so constantly to the mechanical influences of mastication. The skull continues to grow along the lines of suture until these close. The suture lines in the skull correspond to some degree to the epiphyseal plates of the tubular bones.[4]

This summary of the embryogenesis and postnatal development of the skeleton shows that bone formation and growth proceed at different rates in the various bones, and even in various portions of the same bone. In certain sites very active formation of bone occurs for a long period, as, for instance, in the metaphyses of the long tubular bones, the costochondral junctions, the jaw and the vicinity of the sutures of the skull bones. The junctions of cartilage and bone in the epiphyses, especially of the long tubular bones, are sites of somewhat slower but active formation of bone. When skeletal maturity is reached the growth of the bone ceases, and there is a slowing down of the rate of formation of bone at the sites at which it was previously most active. However, a number of these sites of early active formation and transformation of bone remain capable of again resuming these processes, even during adult life. In regard to the shafts of long tubular bones, it is of interest to observe that in early postnatal life the periosteum is of very great importance in the formation of bone, but as the animal becomes older there is considerable diminution in the functional capacities of the periosteum, which becomes dormant.

EXPERIMENTAL RESULTS

Decalcification is the only established specific effect of parathyroid extract (parathormone) on bone, and the resorption is generally evidenced by the presence of enlarged haversian canals or by lacunae containing osteoclasts on the periosteal and endosteal surfaces of bone. It is therefore possible to follow by histologic methods the complete anatomic distribution of the changes induced by such treatment. A young guinea-pig, weighing 300 Gm., into which 20 units of parathyroid extract per hundred grams of body weight has been injected, will show in forty-eight hours the greatest amount of resorption at the sites of most active growth of bone, that is, in the metaphyses of the long tubular bones, at the costochondral junctions, in the shaft cortices (most intense at the

3. Dawson, A. B.: Anat. Rec. **31**:1, 1925.

4. Mair, R.: Ztschr. f. d. ges. Anat. (Abt. 1) **90**:293, 1929.

encoche or terminal portion of the cortex and diminishing as the cortex of the middle of the shaft is approached). The jaw shows intense decalcification and resorption.

In the study of the bones of a large number of animals suffering from experimental hyperparathyroidism and showing consistently the histologic changes described, we were impressed by the equally consistent absence of these changes in spongy bone [5] of the epiphyses of the long tubular bones. We therefore undertook to determine whether changes could be induced in the epiphyses and in bones formed like the epiphyses in guinea-pigs weighing 300 Gm. by the injection of much larger amounts of parathormone.

Three guinea-pigs of this weight were given single doses of 50, 100 and 165 units of parathyroid extract per hundred grams of body weight. Extensive resorption, with the presence of osteoclasts and Howship's lacunae, was found at the

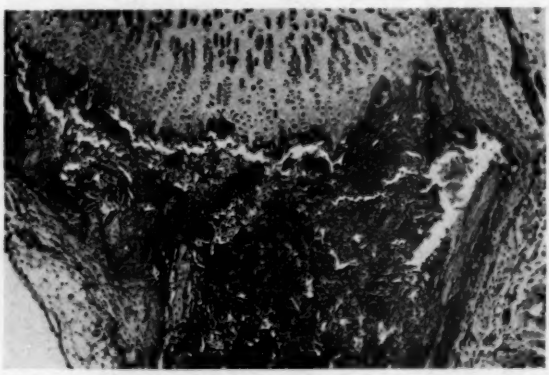

Fig. 1.—The costochondral junction of a rib of a young guinea-pig weighing 300 Gm. The animal was killed forty-eight hours after the injection of 500 units of parathyroid extract. Figures 1 to 6 all represent effects produced in this animal. Here it is to be noted that the cortex is fractured and the bone disintegrated, that the columns of endochondral bone are splintered, that new endochondral ossification has ceased, and that there is considerable hemorrhage in the marrow; × 40.

5. Roux (Die Entwicklungsmechanik, Vortr. u. Aufs. ü. Entwcklngsmech. d. Organ., 1905, nos. 1 to 5) classified the components of spongy bone as follows: (1) tubes (tubuli), (2) hemispheric spaces (pilae), (3) plates (lamellae) and (4) beams (trabeculae). The tubuli ossei are the long spaces in spongy bone enclosed by the thicker trabeculae, and the pilae ossei are curved spaces in spongy bone, particularly underneath the articular cartilage, and are enclosed by the thinner lamellae. Any classification of the elements of spongy bone must be a rough one, because there are innumerable transition forms and shapes that make a strict terminology impossible (Jaffe, H. L.: Structure of Bone with Particular Reference to Its Fibrillar Nature and Relation of Function to Internal Architecture, Arch. Surg. **19**:24, 1929). The term "trabeculae" is frequently used as a synonym for the term "spongy bone."

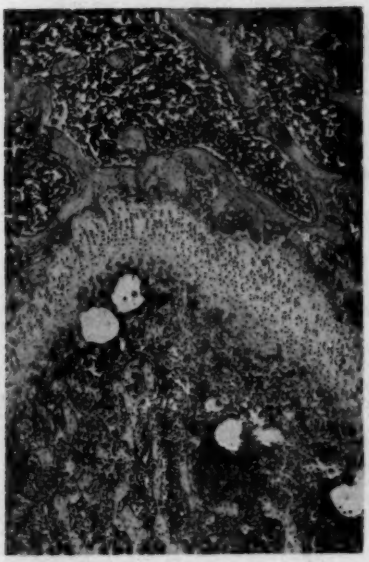

Fig. 2.—The lower end of the femur; × 22. There are considerable resorption of bone and fibrosis of marrow in the metaphysis; a number of small cysts are present beneath the epiphyseal cartilage plate. No such changes are observed in the epiphysis above the plate, where the marrow is normal.

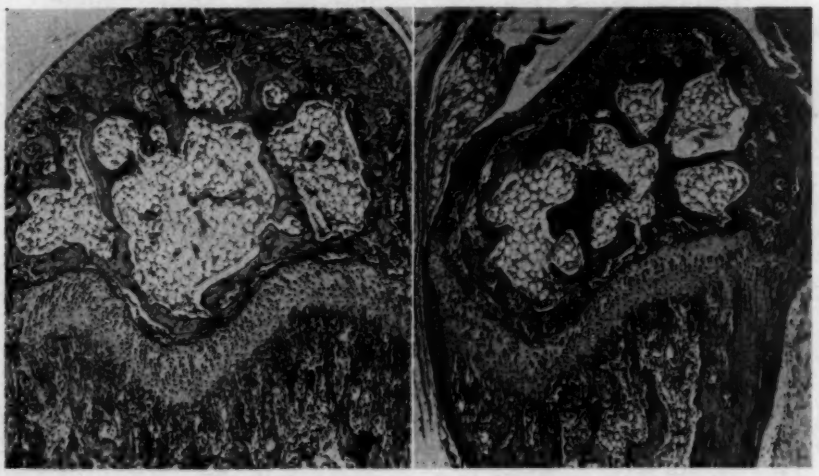

Fig. 3.—At the left are the epiphysis and part of the metaphysis of a metatarsal bone; × 22. The marrow of the epiphysis is fatty and no abnormal resorption is observed. Some fibrosis of the marrow and osteoclastic resorption of the bony trabeculae in the metaphysis is seen, but the changes are less than in the corresponding region in figure 2.

At the right are the epiphysis and the metaphysis of a phalanx; × 22. The epiphysis appears normal, but the metaphysis shows some osteoclastic resorption. The changes are less than in the metatarsal at the left.

costochondral junctions, in the metaphyses, in the shafts of the ribs and rapidly growing long tubular bones, and in the skull and jawbones. However, the spongy bone of the epiphyses and of the carpal and tarsal bones of these animals showed a striking absence of change. At most, it showed only simple thinning of the elements.

The shaft cortices showed an amount of resorption directly proportional to the growth capacity of the bones, the cortices of the ribs and long tubular bones showing much resorption, and those of the metacarpal, metatarsal and phalangeal bones little or none. In two of these young guinea-pigs, the endochondrally formed patella showed evidences of active resorption following the injection of 100 and 165 units of parathyroid extract per hundred grams of body weight. The reason

Fig. 4.—At the left is the encoche or terminal portion of the cortex of the shaft of the lower end of the femur; × 85. Note the large number of osteoclasts and the connective tissue in the enlarged canals of the blood vessels. Resorption is very active, as this is an area of rapid growth. At the right is the encoche or terminal portion of the cortex of the shaft of a phalanx; × 85. Note that resorption and fibrosis are absent here. Compare with the corresponding region in the rapidly growing bone at the left.

for this probably lies in the fact that the patella does not begin to ossify until relatively late and, because of the active progress of ossification, responds more like the spongy bone of the metaphyses than like that of the epiphyses and of the tarsal or carpal bones (figs. 1 to 6).

We also studied the distribution of lesions in the bones of young guinea-pigs receiving repeated, stepped-up doses of parathyroid extract over a period of twenty days.

Six guinea-pigs, weighing between 270 and 320 Gm., received injections of gradually increased doses of parathyroid extract, finally receiving 60 units daily for the last four days. The distribution of the lesions was the same as in the

young guinea-pigs receiving 1 large dose. No osteoclastic resorption or fibrous tissue formation was found in any of the epiphyses or in the carpal and tarsal bones. The shafts of the slowly growing short tubular bones showed less resorption when compared with the shafts of the rapidly growing long tubular bones, and the lesions in the metaphyses were less prominent than in the long tubular bones.

In line with the finding that the resorption effects of parathyroid extract are most marked in those portions of the skeleton that are most

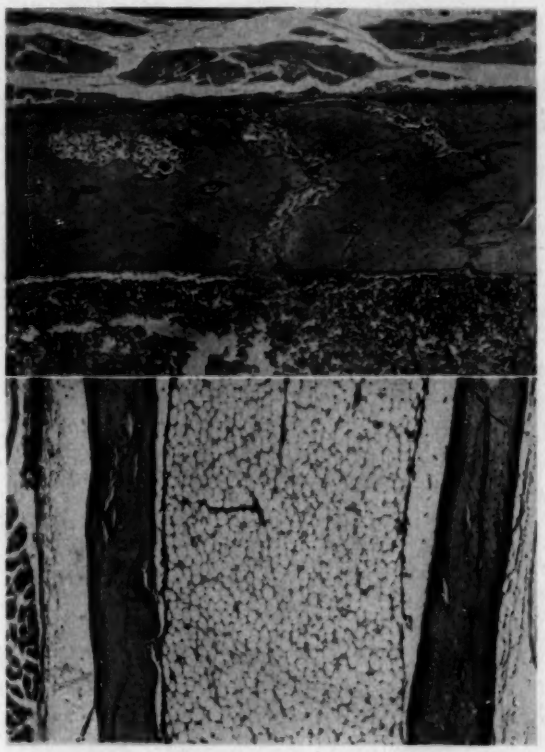

Fig. 5.—Above is a section through the cortex of the middle of the shaft of the humerus; × 85. Note the active subperiosteal resorption and the enlargement of the canals of the blood vessels. A Volkmann canal is observed penetrating the entire thickness of the cortex.

Below is the cortex of the shaft of a metacarpal bone; × 85. The bone is compact and resorption is not observed. Compare with the cortex of the rapidly growing bone above.

actively growing is the observation that the bones of adult guinea-pigs are relatively much less susceptible to the effects of a single dose of parathyroid extract than those of young animals.[6] However, when very

6. Jaffe, H. L.; Bodansky, A., and Blair, J. E.: Fibrous Osteodystrophy (Osteitis Fibrosa) in Experimental Hyperparathyroidism of Guinea-Pigs, Arch. Path. **11**:207, 1931; J. Exper. Med. to be published.

great doses are administered to adult guinea-pigs, resorption may be found where formation of bone still occurs, though this is reduced to a state of slight activity. Thus, if the encoche is still present, resorption and fibrosis will be found in it. Since maturity of the guinea-pig is accompanied by a diminution in the number of the haversian canals in the shaft cortices, resorption, when it occurs in the cortices, appears beneath the endosteum and periosteum, but mostly beneath the endosteum. It is actually to the greatest degree beneath the endosteum, which in adult life has greater osteogenic capacity in the normal transformation of bone.

Fig. 6.—Part of a section of a tarsal bone; × 85. Note the absence of marrow changes and the absence of resorption of the spongy bone. This bone responds to parathyroid extract like an epiphysis.

Seven adult guinea-pigs received daily injections of parathyroid extract for from seven to eleven days. They received a total of from 200 to 540 units of parathyroid extract during this period. All died from acute hyperparathyroidism. The histologic examination showed that with such treatment changes in bone and marrow could be produced in adults; the degree varied directly with the total dosage of parathyroid extract given, but the adults that were younger showed relatively more changes in bone and marrow, on a given dosage, than those that were older. The lesions, wherever present, although not severe, showed the greatest degree of advancement in the same sites as in the animals receiving large single doses. It should be observed, however, that none of the metacarpal, metatarsal or phalangeal bones showed any subperiosteal or subendosteal resorption. The carpal and tarsal bones, with the exception of the os calcis, showed complete absence of the resorption effects of hyperparathyroidism. The epiphyseal ends of the long

tubular bones showed practically no changes in the marrow or spongy bone. The youngest of these adults showed some osteoclasts and Howship's lacunae on the bone near the articular cartilages.

In three animals, in which care was taken not to increase the dose too rapidly, and to intermit the treatment when they appeared sick from hyperparathyroidism, injections of parathyroid extract were continued for sixty-nine days, with gradually increased dosage. Two of these guinea-pigs received a total of 3,040 units during this period; the third animal received more rapidly increasing doses during the last thirteen days of the treatment, and was given a total of 4,020 units. Bone

Fig. 7.—Above is the cortex of the middle of the shaft of the femur of an adult guinea-pig that received increasing doses of parathyroid extract for sixty-nine days. The animal received 3,040 units. The cortex shows marked enlargement of the canals of the blood vessels, which are filled with connective tissue and show osteoclasts in Howship's lacunae; × 40.

Below is a section through the cortex of the middle of the shaft of a metacarpal bone from the same animal; × 40. No lesions are observed in the cortex of this slow-growing bone. Compare with the femoral cortex above.

changes were produced, characterized by extensive enlargement of the haversian canals, which contained connective tissue and Howship's lacunae with osteoclasts. In these animals, however, resorptive changes appeared in the spongy bone of the epiphyseal ends of some of the long tubular bones and of the short tubular bones,

and in the cortices of these bones, especially those of the long tubular bones. Thus, these adult guinea-pigs were the first that showed any extensive involvement of the epiphyses of either the long or the short tubular bones. The carpal and tarsal bones showed slight evidences of resorption — insignificant compared with the epiphyses (fig. 7).

COMMENT

The experiments described showed that the resorption of bone in hyperparathyroidism occurs in certain sites of predilection. The increased susceptibility of these sites may be related to their rate of bone formation. It may be stated as a general principle that the sites of most active formation of bone are the sites most susceptible to physiologic or pathologic resorption of bone. Thus, those portions of the skeleton most susceptible to decalcification are those in which formation of bone is most active.

As we pointed out in the summary of the embryogenesis and postnatal development of bone, the tarsus, carpus and formed epiphyses of a young guinea-pig are sites of relatively slow growth, and are therefore least susceptible to the effects of hyperparathyroidism. These bones therefore respond to parathyroid extract in a manner similar to that of the bones of the adult skeleton. As the animal becomes older, and full growth in the length of the bones is completed, the bones or portions of bones that are most susceptible to resorption in the young animal become less so. When the animal is fully adult, the entire skeleton, although less susceptible, shows more or less uniformity in the response to the decalcifying effects of parathyroid extract.

On the other hand, Bauer, Aub and Albright [7] stated that in resorption of bone the calcium of the trabeculae [8] is "labile" and is drawn on in the first instance, while the calcium of the compact bone ("the structural parts of the bone") becomes available only "in the case of unsual body demands." They arrived at their conclusions partly through a study of the effect of parathyroid extract (parathormone) on rabbits, kittens and rats. After continued administration of parathyroid extract to three 5 week old rabbits for ninety-one days, during the major part of which (from fifty-six to seventy-four days) the rabbits received 8 units daily, the trabeculae were reported as diminished in number, but no effect was noted on the cortex of the bone either by gross or by roentgen examination. In two kittens 26 days of age, the administration of an average of 25.4 units of parathyroid extract daily for fifty-six days did not result in any greater reduction of the trabeculae than was observed in the course of the growth of bone in the normal controls. Four rats, 10 days of age at the beginning of the experiment, received

7. Bauer, W.; Aub, J. C., and Albright, F.: J. Exper. Med. **49**:145, 1929.

8. Bauer, Aub and Albright use the term "trabeculae" as a synonym for "spongy bone."

885 units each during a period of one hundred and ten days. Roentgen examination disclosed a diminution in length of the bones, but an increase in the number of trabeculae.

In spite of the fact that their data, as given here, do not seem to support their view, Bauer, Aub and Albright concluded that bone trabeculae are easily depleted by prolonged administration of parathyroid extract, and that these trabeculae serve as the source of readily available calcium. To a great degree their conclusions regarding the effects of parathyroid extract seem to have been inferred from the observation that adult cats show a depletion of trabeculae in the metaphyseal regions of the lower end of the femur and the upper end of the humerus following a diet low in calcium, and that following a diet high in calcium there is a reaccumulation of prominent trabeculae in these regions. Furthermore, they based their conclusions in part on the fact that intravital staining with alizarin red during the periods of feeding large amounts of calcium resulted in the deposition of the dye in the newly formed trabeculae. It is also interesting to note that no gross changes in the cortices of the shafts were reported by these authors, although some of their animals were kept on a diet low in calcium for a long period of time.

Regarding the effect of parathyroid extract on the bones of rabbits, we believe that Bauer, Aub and Albright should have taken into consideration the fact that the rabbit is a poor animal in which to follow changes in the spongy bone of the ends of the long tubular bones. Normally, relatively little spongy bone is present in these locations. In addition, a histologic study of the bones of animals is necessary before drawing conclusions regarding the absence of changes in the compact cortex. We have frequently found extensive resorption of cortical bone in our experimental animals on histologic examination, even when the gross examination revealed nothing striking. That this also holds true when roentgen examination of animal bones is employed as the sole means of judging decalcification is obvious. Furthermore, one may question the validity of generalizations concerning the physiologic functions of spongy bone in calcium metabolism, if not based on a histologic study of bones that contain considerable spongy elements, such as the patellae, vertebrae, tarsal and carpal bones, and the epiphyseal ends of the tubular bones.

On the basis of our experimental work, we believe that the widely accepted conception of Bauer, Aub and Albright is not tenable, and that the following formulation of the mechanism that determines the availability of calcium during resorption of the bones is preferable: Bone resorption and bone deposition are processes that go on in all bone constantly. When the rate of either of these processes is increased, it is increased in all bone. Both processes are more rapid in the regions of active growth, irrespective of anatomic structure. A mechanism is

therefore indicated for rapid decalcification in those regions when a condition is present favoring an excess of resorption over deposition. In regions of less active bone growth, resorption and decalcification proceed more slowly under normal conditions, and a great stimulus is required to produce a considerable degree of resorption. Thus, if we are to speak of labile calcium, it is the calcium in the regions of most active bone growth; if we are to speak of less readily available calcium, it is the calcium in the regions of less active growth.

This conception makes intelligible the pronounced susceptibility to resorption of the spongy bone of the metaphyses, especially those of the rapidly growing long tubular bones; of the costochondral junction; of the cortices of the shafts, particularly near the epiphyseal cartilage plates; of the cortices of the ribs used most in the respiratory act, and of the bones of the skull and of the lower jaw. It also explains why the metaphyses of the slower growing short tubular bones show relatively little evidence of resorption, while the cortices of the shafts of these bones show practically no evidence of resorption. At the same time, the spongy bone of all epiphyses and of the tarsal and carpal bones, when subjected to conditions producing the lesions described, show at the most only general thinning. Furthermore, this conception not only makes intelligible the greater susceptibility of the skeletons of actively growing young animals to parathyroid extract, compared with the skeletons of adult guinea-pigs, but explains also the quantitative differences in the response of different bones, and of different portions of given bones in animals treated with parathyroid extract. This is observed strikingly in young animals, but also to a certain degree in older animals.

It is well known that a supply of available calcium increases the hypercalcemic effects of parathyroid extract. We have demonstrated this in dogs under conditions in which the effect of recently absorbed (alimentary) calcium was eliminated.[9] Therefore the greater availability of the bone calcium in young animals is strongly indicated by the greater ease with which hypercalcemia is produced in young animals even of species (like the guinea-pig) which are relatively resistant to the effects of parathyroid extract.[10]

The conception of the quantitative relation of bone resorption to the rate of growth in particular sites may be further extended to include endosteal and periosteal resorption. In young, rapidly growing animals, with the shafts of tubular bones increasing in diameter, the marked degree of subperiosteal resorption that is observed in experimental

9. Bodansky, A., and Jaffe, H. L.: J. Exper. Med. **53**:591, 1931.

10. Bodansky, A.; Blair, J. E., and Jaffe, H. L.: J. Biol. Chem. **88**:629, 1930. Bodansky, A., and Jaffe, H. L.: J. Biol. Chem. **93**:543, 1931.

hyperparathyroidism corresponds to the rapid growth in the subperiosteal regions in the normal animal. In the older animal, in which normally subperiosteal growth is greatly diminished, subperiosteal resorption is correspondingly less marked in hyperparathyroidism, while subendosteal resorption becomes by far the more prominent factor.

While active decalcification with the appearance of osteoclasts is probably the specific effect of parathyroid extract on the bones, this is marked only in the more rapidly growing portions of the bones. In the less rapidly growing portions, as for instance the middle portion of the diaphysis of a long tubular bone, a more generalized phenomenon of thinning is observed. We believe that this thinning underlies all resorption, in hyperparathyroidism as well as in osteoporosis, and affects the compact as well as the spongy bone. It is an expression of the tendency to spread an inadequate quantity of calcium over the entire mass of bone. This tendency is related to the growth stimulus, which is seen especially in the remarkable longitudinal growth of bone in spite of a calcium deficiency. This calcium deficiency may be of dietary origin or may be produced by such agents as parathyroid extract. The picture thus produced is analogous to the "smooth bone resorption," which was recognized by the earlier pathologists.[11]

In harmony with our view is also the old observation of Schmorl,[12] who in 1909 stated, in a discussion of clinical rickets, that the lesions appear earliest and are most severe in the regions of rapid growth, and the observation of many workers that experimental rickets is most easily produced in the rapidly growing animal.

Regions of rapid growth in bone, in agreement with our view, are also most susceptible to the effects of elementary phosphorus [13] and lead,[14] which cause the formation of thickets of spongy bone in these regions. The work of Park, Jackson and Kajdi [14] showed that lead, when taken for a sufficiently long period in sufficient dosage, can produce changes in bone, which are most marked where growth is occurring most rapidly, namely, at the anterior ends of the middle six ribs, at the lower ends of the femurs, at the upper ends of the humeri, at the lower ends of the radii and ulnae, and at both ends of the fibulae and tibiae. In two children, the bone changes produced by lead were well marked in the

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11. Jaffe, H. L.: Resorption of Bone; Consideration of Underlying Processes Particularly in Pathologic Processes, Arch. Surg. **20**:355, 1930.

12. Schmorl, G.: Verhandl. d. Gesellsch. f. Nat. u. Heilk., 1908-1909, vol. 90.

13. Wegner, G.: Virchows Arch. f. path. Anat. **61**:44, 1847; **55**:11, 1872. Phemister, D. B.: Effect of Phosphorus on Growing Normal and Diseased Bones, J. A. M. A. **70**:1737, 1918.

14. Park, E. A.; Jackson, D., and Kajdi, L.: Shadows Produced by Lead in X-Ray Pictures of Growing Skeleton, Am. J. Dis. Child. **41**:485, 1931.

roentgenograms of the fast-growing parts of the skeleton, but were not evident in those of the slow-growing parts. The authors concluded that while "growth in bone occurs in many places, only where growth occurs rapidly do the changes readily reach such magnitude as to show in X-ray photographs."

It is suggested that the same principle applies in experimental scurvy, which shows the so-called characteristic lesions in the regions of active growth; the rest of the skeleton of young guinea-pigs on a scurvy-producing diet generally shows the nonspecific effect of the deficiency in the form of various degrees of thinning (simple atrophy). We reviewed the bones of young guinea-pigs suffering from experimental scurvy,[15] and found that the specific lesions were generally confined to the metaphyses, to the cortices of the shafts of the long tubular bones, and to the costochondral junctions. The spongy bone of the epiphyses, at the most, showed simple atrophy. However, if a 200 Gm. guinea-pig was placed on a scurvy-producing regimen, and if severe scurvy developed rapidly, lesions were also occasionally noted in the epiphyses.

It is interesting that a number of clinical diseases of bone occur in the epiphyses or in bones that are formed like the epiphyses. These are the so-called osteochondridites—Perthes' disease, Osgood-Schlatter's disease, Kienböck's disease, Köhler's disease, etc. It is probable that their long clinical course and their slow tendency to heal are related to the localization of lesions in bones (and in regions of bones) of slow metabolic exchange and therefore of slow reparative capacity.

CONCLUSIONS

From a study of the bones of guinea-pigs with experimental hyperparathyroidism we conclude that the rate of decalcification of the bony skeleton at any site is related to the rate of bone growth and metabolic exchange at that site. Decalcification and reactive lesions are more prominent in experimental hyperparathyroidism in regions of normally active growth of bone and less prominent in regions of less active growth. In the latter, simple thinning of the bone is sometimes the only change observed.

This conception makes intelligible the greater susceptibility of young animals to parathyroid extract and the reported details of the changes in the different bones and in the various portions of certain bones in experimental hyperparathyroidism.

15. Jaffe, H. L.: J. Infect. Dis. **40**:502, 1927.

EFFECTS OF INTRATHECAL ADMINISTRATION OF MERCUROCHROME-220 SOLUBLE AND OF METAPHEN *

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Intrathecal injections of dyes and other chemicals have long been used in anatomic and physiologic studies. Quincke,[1] for instance, injected mercuric sulphide intrathecally, Reiner and Schnitzler [2] potassium ferrocyanide, Hill [3] methylene blue and Lewandowsky [4] sodium ferrocyanide, in studying the circulation of the cerebrospinal fluid. It is only recently, however, that the intrathecal injection of chemicals has been tried as a therapeutic measure in such conditions as syphilis, epilepsy and meningitis.

A variety of antiseptics have been studied both for their efficacy in sterilizing the meninges and for their toxicity. Seager [5] used compound solution of cresol in human subjects in 1902, and Wolff [6] in 1915 tried strong silver protein. Flexner and Amoss [7] in 1916 used both compound solution of cresol and strong silver protein in monkeys and found them to be harmful. They concluded that not much can be expected from the chemical treatment of epidemic meningitis and therefore pinned their faith on an immunologic attack; i. e., specific serums.

Flateau and Handelsman,[8] in 1916, employed methenamine, Chinese india ink, a colloidal suspension of silver, silver nitrate, formaldehyde and iodine, intrathecally, all of which they found to be harmful.

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1. Quincke, H.: Arch. f. Anat. u. Physiol., 1872, p. 153.

2. Reiner, M., and Schnitzler, J.: Abstr., Centralbl. f. Physiol. **8**:684, 1894.

3. Hill, L.: Physiology and Pathology of the Cerebral Circulation, London, 1896.

4. Lewandowsky, M.: Ztschr. f. klin. Med. **40**:480, 1900.

5. Seager: Quoted by Flexner and Amoss (footnote 7).

6. Wolff, G.: Deutsche med. Wchnschr. **41**:1486, 1915.

7. Flexner, S., and Amoss, H. L.: J. Exper. Med. **23**:683, 1916.

8. Flateau, E., and Handelsman, J.: Ztschr. f. d. ges. Neurol. u. Psychiat. **31**:1, 1916.

Wegeforth and Essick,[9] in 1919, working on cats with experimentally produced meningitis, employed acriflavine, potassium permanganate, potassium biniodide, phenol, silver nitrate, benzoyl alcohol, mercuric iodide and compound solution of cresol. Continuous lavage, intermittent lavage and simple injection of the drug were tried. None of these drugs was devoid of toxic effects, even when used in low concentrations.

Kolmer,[10] in 1926, reported using ethylhydrocupreine hydrochloride, mercurochrome-220 soluble, metaphen, gentian violet, acriflavine and rivanol in the treatment of pneumococcus meningitis in dogs, with disappointing results. Stewart,[11] in 1927, used ethylhydrocupreine along with serums and lavage in the treatment of pneumococcus meningitis in dogs, with encouraging results. Moldenschardt,[12] in 1929, reported the use of colloidal silver in purulent meningitis. Many other antiseptics have been tried, and all of them have been found to cause a meningeal reaction. This fact, per se, however, constitutes no objection to the use of chemicals intrathecally, since even serums cause a similar reaction, the so-called "serum meningitis." In fact, Goldman,[13] in 1930, found that even the common preservative used in serums, cresol U. S. P., caused a definite though not a severe pleocytosis when injected intrathecally in its commonly employed concentration (0.4 per cent).

Of the current chemical antiseptics, mercurochrome-220 soluble and metaphen have received the most attention in recent literature as nontoxic effective germicides. We therefore deemed it advisable to investigate the toxicity of these drugs injected intrathecally, with a view toward employing them later in the treatment of meningitis. Our problem was (1) to determine their intrathecal sublethal and subtoxic dosages, (2) to study the morbid anatomy following such injections, and (3) to determine whether intrathecally tolerated doses of these chemicals were bactericidal or efficacious in sterilizing the subarachnoid space.

TECHNICAL CONSIDERATIONS

The work reported here was all done on dogs. Because of the smaller size of the subjects and because of certain anatomic differences, subthecal punctures in dogs are generally more difficult to do and more

9. Wegeforth, P., and Essick, C. R.: J. Pharmacol. & Exper. Therap. **13**:335, 1919.

10. Kolmer, J. A.: Pneumococcus and Streptococcus Meningitis, J. A. M. A. **92**:874, 1929; Chemotherapy and Serumtherapy of Pneumococcus and Streptococcus Meningitis; Résumé of Present Status of Treatment of Septic Meningitis, with Recommendation of Method, Arch. Otolaryng. **3**:481, 1926.

11. Stewart, F. W.: Local Specific Treatment of Experimental Pneumococcic Meningitis, J. A. M. A. **89**:1316, 1927.

12. Moldenschardt, H.: München. med. Wchnschr. **76**:1875, 1929.

13. Goldman, D.: Serum Meningitis, Arch. Path. **9**:1027, 1930.

fraught with danger than those in man. Ochsner, Gage and Cutting [14] stated that it is virtually impossible to perform successful lumbar puncture on the dog. We have not found this to be the case, but we must admit that the operation calls for a great degree of skill, is somewhat uncertain, and is frequently attended by injury to the animal. Knowledge of the anatomy involved is therefore essential before attempting subthecal punctures on this animal.

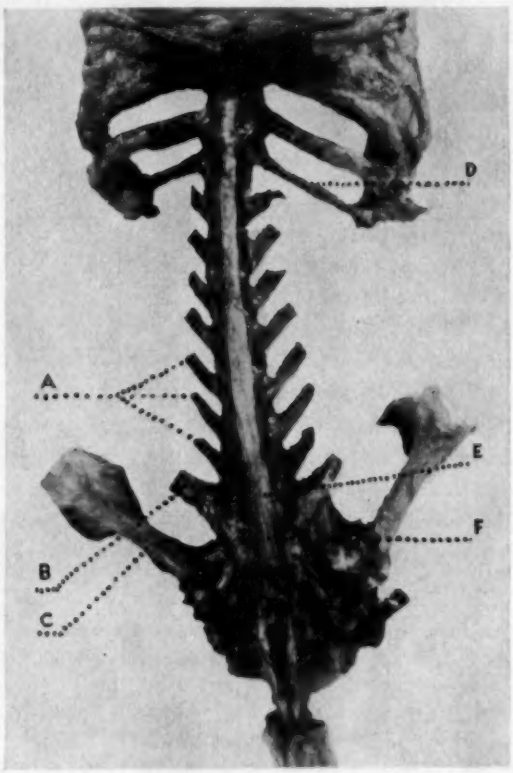

Fig. 1.—The anatomic relations of the lumbar cord: *A*, fifth, sixth and seventh lumbar processes; *B*, iliac crest; *C*, femur; *D*, last rib; *E*, end of cord, and *F*, cauda equina. The top of the vertebral column has been removed, exposing the cord, which extends to the level of the seventh lumbar vertebra. Note the relation of the iliac crests to this level.

In the dog there are, as a rule, seven lumbar vertebrae (fig. 1) and occasionally only six, as compared to five in man. The spinous processes

14. Ochsner, A.; Gage, I. M., and Cutting, R. A.: Comparative Value of Splanchnic and Spinal Analgesia in Treatment of Experimental Ilieus, Arch. Surg. **20**:802, 1930.

are directed slightly cephalad instead of caudad as in man. The cord extends down to the level of the body of the last lumbar vertebra, instead of to the second lumbar vertebra as in man, narrowing rapidly into the conus at the level of the sixth or the seventh lumbar vertebra. The cauda equina extends down to the lower level of the sacrum and is much shorter than in man.

An imaginary line drawn through the iliac crests intersects the lumbar column at the sixth interspace. Lumbar punctures can be performed through this space, the space above it or the space below it; i. e., through the fifth or the sixth lumbar, or through the lumbosacral interspace. Punctures above this level are inadvisable, because of the danger of injuring the cord.

The dura in the lower lumbar region is more loosely adherent to the bony wall (because of the relatively thicker pad of epidural fatty tissue) than in the upper lumbar and thoracic regions, and yields easily before the incoming needle, with the result that a dural snap may not be felt when the needle enters the canal. For this reason, also, dry taps are not infrequent, as the dura may "give" and not be pierced by the needle. A similar condition in the human new-born infant and prematurely born infant has been described by Glaser [15] to explain the difficulty in obtaining fluid by lumbar puncture in these infants.

The low extent of the cord accounts for the frequency of bloody taps and also for the occasional occurrence of a transverse myelitis following simple lumbar puncture.

To keep the animal quiet and thus to minimize the danger of injuring the cord an anesthetic should be used. We employed morphine-ether anesthesia.

For lumbar puncture, a short bevel needle about 7 cm. in length and 22 gage, fitted with an obturator, was used. The anesthetized animal was placed on its left side, the back flexed, and the needle inserted into the sixth lumbar interspace just to one side of the midline and directed forward, cephalad and medially, at an angle of about 45 degrees with the frontal plane, 30 degrees with the sagittal plane, and 45 degrees with the transverse plane, being guided by the body of the spinous process till the posterior arch was reached. As has been pointed out by Dvorak and Manson,[16] the lumbar vertebrae in the dog are too closely approximated to allow a passage of the needle between them in the midline, except in the lumbosacral space. Laterally, however, the interspinous space is amply wide. Through this lateral aperture, gentle probing being used, if necessary, the needle reaches the subdural space usually about from 3 to 5 cm. from its point of entrance into the skin. The cerebrospinal fluid usually trickles slowly out of the needle; occasionally it will gush out, and at other times it will slowly drip out.

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15. Glaser, J.: Cerebrospinal Fluid of Premature Infants, with Special Reference to Intracranial Hemorrhage and Pigmentation, Am. J. Dis. Child. **36**:195, 1928.

16. Dvorak, H., and Manson, M. H.: Proc. Soc. Exper. Biol. & Med. **28**:344, 1930.

For cisternal puncture, a needle about 5 cm. in length and about 22 gage, fitted with an obturator, was used. In doing cisternal punctures, we prefer the lateral position of the dog, the same as for lumbar puncture, to the prone position used by Goldman.[18] The occipital protuberance was palpated with the index finger and the protuberance of the axis with the thumb of the left hand, and the needle was inserted midway between these points, being directed upward and forward at an angle of about 45 degrees, and directly into the cistern.

The skull from the occipital protuberance to the border of the foramen magnum, in the dog, slants more obliquely forward than in the human being. This should be kept in mind if the indirect method of cisternal puncture—that is, feeling one's way along the skull with the needle—is used. The cistern in the dog is relatively smaller than that in the human being, so that injury to the medulla is much more likely to occur. The cervical cord extends for a distance of about from 0.5 to 1 cm. into the cranial cavity, so that if the needle is not directed obliquely forward and upward, injury to the cervical cord may ensue. In spite of all precautions, however, bloody fluids are not uncommon following lumbar or cisternal puncture.

In addition, we have observed the following clinical symptoms and sequelae attending simple lumbar puncture:

1. Jerking of the feet or tail when the cord or nerves were touched by the needle.
2. Listlessness, anorexia, asthenia, ataxia and hyperesthesia of the lower half of the body for twenty-four hours after the puncture.
3. Transverse myelitis due to injury of the cord.
4. Subarachnoid block, causing subsequent dry taps, arachnoid adhesions being found post mortem.
5. Local subcutaneous hematoma at the site of puncture.

Subsequent to cisternal puncture, the following sequelae have been noted:

1. Death due to puncture of vital centers in the medulla.
2. Respiratory embarrassment, i. e., dyspnea, apnea and Cheyne-Stokes' respiration, due to hemorrhage about the cistern.
3. Asthenia, ataxia, tremors, irritability, loss of equilibrium and anorexia for twenty-four hours after the puncture.

Since the introduction intrathecally of very small amounts of material can enormously raise the intracranial pressure, the following precautions were observed when such injections were made:

The solution was injected at a temperature of about 40 C., and at a pressure of from 20 to 25 cm. water. Pressure readings before and during the injection were taken by means of a manometer that was attached either to a two-way stopcock connected to the puncture needle or directly to a second needle placed into the subarachnoid space for this purpose.

When cistern-lumbar lavage was performed, the temperature of the solution was controlled by means of a simple apparatus described by one of us.[17] The pressure was controlled by raising or lowering the reservoir containing the solution, since the fluid was allowed to flow in by gravity. In order to avoid injury to the medulla due to movement of the cisternal needle, we used a combination guard and retainer on the cisternal needle, whenever cistern-lumbar lavage was done.

Necropsy.—The removal of the cord and brain intact in a dog is a tedious, but frequently a necessary, procedure. After experimenting with several methods of performing this part of the necropsy, such as sawing off the top of the vertebral column and skull, using a double saw rachitome to divide the transverse processes, using trephine openings in removing the skull and several other variations, we evolved the following technic, which we believe to be the simplest, quickest and most efficient. By this method it is possible for one person to remove the brain and cord intact in from fifteen to twenty minutes.

The dog's body is placed prone on a flat table. The skin is split the whole length of the back from the snout to the tail and then the subcutaneous tissues and muscles are incised along the whole length of the vertebral column, the knife passing along each side of the spinal column in the groove between the spinous processes and the uppermost projections of the transverse processes. The temporal muscles are dissected away from the skull. The transverse processes on each side of the spine are then divided by means of a single blade rachitome. The blade is placed in the groove on either side of the spinous processes and is sharply struck with a heavy wooden mallet. Then, with the use of a sequestrum or bone forceps, the top of the vertebral column can easily be lifted off by starting at the sacrum and ripping upward to the skull. The remaining edges of the bone can be trimmed, if necessary, with a bone-cutter. The skull is then split by three blows, one with the rachitome blade placed transversely just posterior to the frontal sinuses and the other two with the blade placed successively on each side of the skull, in the temporal fossae parallel to the sagittal suture and about midway between the midline and the external auditory meatus. The skull can then be lifted off, leaving only a centimeter or so of thin bone over the medulla, which can easily be removed with a bone-cutter. The spinal cord with its dura and the brain are then removed intact in retrograde fashion by beginning with the cauda and dissecting upward. The cord and brain may then be fixed in formaldehyde pending further examination.

MERCUROCHROME

Toxicity.—We attempted to determine the maximum tolerated doses of mercurochrome-22 soluble, H. W. & D. (disodium-monohydroxy-mercuri-dibromfluorescein) both by intraspinal and intracisternal injection in dogs. At the start we were unaware of the great toxicity of this drug when injected intrathecally. It soon became apparent that the usual 2 per cent or even the 1 per cent mercurochrome was too toxic. Therefore, we resorted to the use of 0.25, 0.10 and 0.025 per cent solu-

17. Perlstein, M. A.: J. Lab. & Clin. Med. **16**:837, 1931.

tions (1:400, 1:1,000 and 1:4,000). The solution was injected slowly by means of a syringe, being continuously mixed with cerebrospinal fluid by a process of sufflation during the course of the injection.

Table 1 indicates the great toxicity of mercurochrome when injected into the cistern of a dog. Of seven dogs, in all of which there was no postmortem evidence of hemorrhage or trauma due to the puncture itself, only two failed to manifest any toxic symptoms. These two dogs received the smallest doses, 0.055 and 0.066 mg. per kilogram, respec-

TABLE 1.—*Summary of Experiments with Intracisternal Injection of Mercurochrome in Dogs*

Dog*	Weight of Dog, Kg.	Amount and Concentration of Solution Injected	Amount Mercurochrome Injected, Mg.	Amount Mercurochrome per Kg., Mg.	Results	Structural Changes
1	4.5	1 cc. of 0.025% (1:4,000)	0.25	0.055	No toxic symptoms; complete recovery; dog had mange and died 1 wk. later	No stain left; hyperemia of cord and brain
2	25.0	1.65 cc. of 0.10% (1:1,000)	1.65	0.066	No toxic symptoms; complete recovery; dog killed 13 days later	No stain left; dilated central canal; otherwise normal
3	10.9	1.2 cc. of 0.10% (1:1,000)	1.2	0.110	Listlessness for 24 hrs.; complete recovery; dog killed on seventh day	No stain left; organizing serous leptomeningitis
4	17.2	1 cc. of 0.25% (1:400)	2.5	0.145	Dog died respiratory death within 35 minutes	Stain covers all of cord and base of brain; very little over cortex and none in lateral ventricles; otherwise grossly normal
5	6.5	1 cc. of 0.10% (1:1,000)	1.0	0.154	Toxic symptoms (Cheyne-Stokes' respirations, convulsions, etc.) lasting 1 hr.; complete recovery	(Dog ran away six weeks later)
6	16.2	1 cc. of 1% (1:100)	10.0	0.616	Dog died respiratory death with convulsions within 12 min.	Stain covers cord and base of brain, but not cortex or ventricles; moderate hyperemia
7	14.1	2.5 cc. of 2% (1:50)	50.0	3.542	Dog died respiratory death within 5 min.	Stain as in dog 6; hyperemia

* One dog is omitted because postmortem evidence of trauma to the medulla was found.

tively. One that received 0.110 mg. per kilogram was listless for twenty-four hours, but made a complete recovery. Another that had received 0.154 mg. per kilogram underwent a severe reaction, hovering on the lethal, but finally recovered without residual ill effects. In the other animals that received 0.145 mg. per kilogram or more, death ensued within from five to thirty-five minutes, depending on the dosage employed.

The minimum lethal dose of mercurochrome, when injected into the cistern of the dog, would therefore be between 0.110 and 0.145 mg. per kilogram, while the maximum subtoxic dose would be between 0.066 and 0.110 mg. per kilogram.

When injected into the lumbar spine (table 2), the drug was less fatal than when injected into the cistern. In fact, a dose as high as 1.21 mg. per kilogram, the highest dose used, failed to kill the animal. Nevertheless, it was very toxic, the subtoxic dose being 0.17 mg. per kilogram. Of five dogs receiving 0.22 mg. per kilogram or more of mercurochrome intraspinally, all manifested reactive symptoms, such as

TABLE 2.—*Summary of Experiments with Intraspinal Injection of Mercurochrome in Dogs*

Dog	Weight of Dog, Kg.	Amount and Concentration of Solution Injected	Amount of Mercurochrome, Mg.	Amount of Mercurochrome per Kg. Mg.	Results	Structural Changes
1	14.3	1 cc. of 0.10% (1:1,000)	1.0	0.07	No toxic symptoms; complete recovery; dog killed 11 wk. later	Small subdural hemorrhage in lumbar cord; otherwise normal
2	8.8	1.5 cc. of 0.10% (1:1,000)	1.5	0.17	No toxic symptoms; complete recovery; dog killed 1 mo. later	Hyperemia of cord and brain; organizing serous meningitis
3	7.3	1.6 cc. of 0.10% (1:1,000)	1.6	0.22	Transverse myelitis developed, which improved so that on 11th day dog had only slight limp; killed on 11th day	Organizing serofibrinous meningitis of cord in lower thoracic to sacral region
4	10.4	1 cc. of 0.25% (1:400)	2.5	0.24	Immediate mild general reaction; transverse myelitis which did not improve in 6 wk., at which time dog was killed	Lumbar myelomalacia
5	9.1	2 cc. of 0.20% (1:500)	4.0	0.44	Transverse myelitis, which cleared up at end of 1 wk.; dog killed on 11th day	Focal myelomalacia of lumbar cord; organizing edema of lumbar subarachnoid space
6	10.0	1.1 cc. of 1% (1:100)	11.0	1.10	No transverse myelitis; dog gave birth to litter of pups on 7th day; was very weak and irritable, and lost weight; killed on 11th day	Acute fibrinopurulent meningitis
7	16.5	2 cc. of 1% (1:100)	20.0	1.21	Immediate convulsions and dyspnea; attack lasted 40 min.; transient palsy of upper extremity; transverse myelitis of lumbar cord, which did not improve; dog killed 1 mo. later	Lumbar myelomalacia

anorexia, ataxia, irritability and tremors. In addition, four of these showed clinical symptoms of transverse myelitis. We are convinced that the transverse myelitis in all of these animals was not the result of faulty technic, but due rather to the action of the drug proper, since there were no postmortem evidences of mechanical trauma, and since the incidence of transverse myelitis following simple lumbar puncture or following the injection of substances other than mercurochrome was much less, in our experience, than following the injection of mercurochrome. The maximum subtoxic dose of mercurochrome intraspinally, as evidenced in our work, is therefore between 0.17 and 0.22 mg. per kilogram.

The relatively greater toxicity of mercurochrome when injected intrathecally than when given intravenously had been previously pointed out by Kolmer [18] and by McKinley and Holden.[19] Kolmer determined the maximum single tolerated dose of mercurochrome for dogs by intravenous injection to be 35 mg., corresponding to 3.5 cc. of a 1 per cent solution per kilogram. When given intrathecally, he found the maximum single tolerated dose to be between 1 and 3 mg. per kilogram. In other words, he found that mercurochrome was from 12 to 35 times more toxic when injected intraspinally than when injected intravenously. These figures were all for normal dogs. In dogs suffering from meningitis, the intrathecal tolerance for mercurochrome, according to Kolmer, was even less, the maximum tolerated dose for repeated injections being between 0.1 and 0.3 mg. per kilogram, or about one tenth of that of a normal dog.

The figures given by Kolmer for the maximum tolerated dose of mercurochrome in normal dogs are about 10 times as high as those found by us. There is a possibility that the mercurochrome we used contained some impurity that might account for its greater toxicity. This is not surprising in view of the results of the chemical analyses of four commercial samples of mercurochrome by Eyre, Notton and Pope.[20] These observers found that whereas the percentage of mercury in the dry material should theoretically be 26.7 per cent, it was as high as 40.9 per cent in one sample and 11.4 per cent in another. Macht and Harden [21] found that the disodium salt of *di*hydroxymercuri-dibromfluorescein that was found as an impurity in the earlier batches of mercurochrome manufactured was much more toxic (by intravenous injection) than mercurochrome (disodium salt of *mono*hydroxymercuri-dibromfluorescein) itself. Burn and Elphick [22] tested eight commercial samples of mercurochrome for their toxicity to mice by intravenous injection, and found that the most toxic sample was over 3.5 times as toxic as the least toxic. It is to be expected that such differences in toxicity would be even greater when the drug is injected intrathecally.

We did not attempt to check this point, for we were primarily interested in the action of the mercurochrome that was used in our hospital

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18. Kolmer (footnote 10, second reference).

19. McKinley, E. B., and Holden, M.: Dangers Attending Intraspinal Treatment with Mercurochrome, J. A. M. A. **88**:1391, 1927.

20. Eyre, J.; Notton, H. E. F., and Pope, W. J.: Brit. M. J. **3**:238, 1928.

21. Macht, D. I., and Harden, W. C.: J Pharmacol. & Exper. Therap. **32**:321, 1928.

22. Burn, J. H., and Elphick, G. K.: Quart. J. Pharm. & Pharmacol. **3**:177, 1930.

service and in our laboratories, and because, in any event, it was quite evident that even the least toxic mercurochrome was too toxic a drug to be used intrathecally.

The greater toxicity of mercurochrome when injected intracisternally than when injected intraspinally is to be expected because of the proximity of the vital centers to the point of injection in the cisternal route. Kolmer's statement that the same dosages of mercurochrome are tolerated by intracisternal or intraventricular injection as by intraspinal injection has not been borne out by our work.

Our figures for the single maximum subtoxic doses in normal dogs are about the same as those given by Kolmer for repeated injections in dogs with meningitis. Since our work with mercurochrome to date has been done only on normal dogs, we have been unable to check his reported greater toxicity of the drug in the presence of meningitis. However, it is reasonable to expect that such a difference exists, because of the damaged condition of the meninges in meningitis. The lowered sugar content of the cerebrospinal fluid in purulent meningitis may also be a factor in the greater toxicity of the drug in the presence of a meningitis, in view of the observation by Macht and Harden [21] that by intravenous injection mercurochrome is less toxic when injected in combination with dextrose than when injected alone.

The only other reference we could find in the literature to the toxicity of mercurochrome for animals by intrathecal injection is by McKinley and Holden, who injected it intraspinally into rabbits. They found that doses as low as 0.4 mg. per kilogram, the lowest that they tried, caused convulsions and death. In the interpretation of these results in animals, it should be remembered, as has been pointed out by Young and his co-workers,[23] that by intravenous injection mercurochrome has the same toxicity for human beings as for rabbits, being slightly more toxic for dogs.

Bactericidal Activity of Mercurochrome in Vitro.—In order to determine whether sublethal doses of mercurochrome would be bactericidal, it was necessary to determine the bactericidal action of various dilutions of mercurochrome. Thalhimer [24] found that dilutions above 1:1,000 were not bactericidal to twenty-four hour broth cultures of Bacterium typhosus in fifteen minute exposures. Kolmer [18] found the range of final bactericidal dilution of mercurochrome in purulent cerebrospinal fluid containing pneumococci and streptococci to vary from 1:400 to

23. Young, H. H.; Hill, J. H., and Scott, W. W.: Treatment of Infections and Infectious Diseases with Mercurochrome-220 Soluble; Analysis of 210 Cases that Furnish Many Definite Examples of Sterilisans Magna, Arch. Surg. **10**:813, 1925.

24. Thalhimer, W.: Personal communication to the authors.

1 : 1,000. Birkhaug [25] found the highest dilution of mercurochrome that would kill Staphylococcus aureus, Streptococcus hemolyticus, B. coli, Gonococcus, B. anthracis and B. subtilis to be 1 : 320. We found the highest dilution of mercurochrome in salt solution that would kill a twenty-four hour broth culture of B. coli after five minutes' exposure to be 1 : 1,000, using one loopful of a suspension of the organism to 0.5 cc. of the chemical. When dilutions were made with cerebrospinal fluid, instead of with salt solution, the results were the same, indicating that the cerebrospinal fluid itself has no significant bactericidal action. Since the maximum sublethal dose of mercurochrome by intracisternal injection is less than 0.145 mg. per kilogram, or for a 10 kilogram (22 pound) dog, 1.45 cc. of a 1 : 1,000 solution or for an adult person weighing 60 kilograms (132 pounds) about 8.7 cc. of a 1 : 1,000 solution or 0.87 cc. of a 1 per cent solution, the futility, as Kolmer has pointed out, of attaining by simple injection a bactericidal action in the subarachnoid space of any therapeutic value is evident.

Lavage.—Since by intraspinal and intracisternal injections such small quantities and concentrations of mercurochrome must be used as are barely bactericidal to begin with, and therefore certainly not bactericidal after dilution in the subarachnoid space, we decided to test out, by lavage from the cistern to the lumbar region, the toxicity of a 1 : 1,000 (0.1 per cent) solution of mercurochrome, that is, the highest dilution that we found to be bactericidal. It was thought that perhaps by this means of withdrawing part of the mercurochrome solution a sublethal bactericidal dose might be maintained in the subarachnoid space. Lavage from the cistern to the lumbar region was therefore performed in four dogs. In all there was a fatal outcome. The protocol of one of these experiments, which is illustrative of the others, follows:

Sept. 26, 1930: Dog P53P was used. The weight of the animal was 14.5 Kg. It was placed under morphine-ether anesthesia, and lumbar and cisternal punctures were performed. Clear fluids were obtained with cell counts of 3 lymphocytes and 1 lymphocyte, respectively (samples A and B, respectively).

At 3 : 37 p. m., treatment was started by lavage from a cisternal to a lumbar needle with 0.1 per cent mercurochrome in physiologic solution of sodium chloride at body temperature at a pressure of 22 cm. of water.

At 3 : 41 p. m., 4 cc. was collected at the lumbar needle, mercurochrome becoming visible in the lumbar fluid.

At 3 : 46 p. m., 9.5 cc. was collected (sample C taken).

At 3 : 50 p. m., 14.5 cc. was collected (sample D taken), fluid running in well.

At 3 : 53 p. m., 21 cc. was collected; the dog's respiration was a little deeper.

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25. Birkhaug, K. E.: Metaphen (4-Nitro-3, 5-Bisacetoxy-Mercuri-2-Cresol: Comparative Study of Commonly Used Disinfectants and Antiseptics; Histologic Changes Produced by Intravenous Administration of Metaphen in Rabbits, J. A. M. A. **95**:917, 1930.

At 3:55 p. m., 28 cc. was collected (sample E taken), respiration becoming more rapid.

At 3:59 p. m., 36 cc. was collected (sample F taken). The breathing was irregular, gasping; the dog's head was kept retracted, and the body was stiff. The cisternal needle was removed twenty-two minutes after the start of the lavage and after 40 cc. of 0.1 per cent mercurochrome had been introduced into the cistern. Anesthesia was stopped.

At 4:01 p. m., 5.5 cc. more was collected from the lumbar needle after the cessation of the introduction of fluid, making a total of 41.5 cc. collected.

At 4:04 p. m., 1.5 cc. more was collected at the lumbar needle, making a total of 43 cc. collected; the fluid was coming very slowly, about 1 drop a minute; the needle was removed from the lumbar canal. The dog's respiration was a little better.

At 4:14 p. m., the animal was coming out of the ether. The head was still kept retracted; the respiration was still a little rapid, but regular.

Summary of Lavage: The elapsed time was twenty-two minutes; the amount introduced, 40 cc.; the amount removed, 36 cc. In the five minutes after stopping lavage, 7 cc. more was collected at the lumbar needle.

Colorimetry: With the use of a Duboscq colorimeter, the specimens taken at various stages during the lavage were compared with the original 0.1 per cent solution of mercurochrome for intensity of color: Sample C showed 26 per cent, sample D 36 per cent, sample E 59 per cent, and sample F 52 per cent, of the original color.

Bactericidal Activity: The last specimen (F) of lavaged fluid was tested for bactericidal action against a twenty-four hour broth culture of B. coli, the cerebrospinal fluid before lavage (sample A) and the 0.1 per cent mercurochrome solution being used as positive and negative controls, respectively. It was found that both the mercurochromized lavaged fluid and the original 0.1 per cent solution were bactericidal to these organisms after fifteen minute exposures. (It should be pointed out that a different strain of B. coli from that to which mercurochrome was found to be bactericidal only in 1:1,000 dilution was used in these tests.)

Sept. 27, 1930: The dog was unable to get up. It had been comatose since lavage. It moved its fore legs, but apparently not its hind legs. Ether anesthesia was given. Lumbar puncture yielded 2 drops of thick, blood-tinged fluid that did not flow through the needle. By cisternal puncture, 1.5 cc. of pinkish fluid was obtained that clotted immediately. Chemical examination of this fluid revealed the total nitrogen to be 0.49 Gm. per hundred cubic centimeters. The total protein was 3.06 Gm. per hundred cubic centimeters. Practically all of the protein consisted of albumin, there being only a minute quantity of globulin present.

Twenty hours after the lavage, the dog was moribund. It was then anesthetized and killed.

Postmortem Examination: A mercurochrome stain was present all over the cord and the base of the brain, but not over the hemisphere nor in the lateral ventricles. There was a moderate hyperemia of the blood vessels over the surface of the cord and brain. There were hemorrhages, varying in size from that of a pinpoint to that of a pinhead and in some places to that of a split pea, scattered throughout the white matter and involving practically all of it, and extending to a smaller degree into the gray matter of the cord, medulla and pons (fig. 2). The changes were greatest in the region of the medulla and cervical cord, gradually becoming less marked above and below this point, so that in the lumbar region there were only one or two such areas of hemorrhage, while the sacral and caudal

areas looked normal. There was a blood clot in the fourth ventricle extending up into the third, but not into the lateral ventricles. In one area it encroached on the cerebellar substance. There were no changes above the level of the optic chiasma, except for the presence of perivascular white streaks in the sulci at the base of the brain (exudate). Histologically, in addition to the areas of hemorrhage in the meninges and in the brain and cord, there was an acute purulent leptomeningitis.

The dilution of mercurochrome in the subarachnoid space is probably in the nature of a defensive mechanism, i. e., an attempt on the part of the choroid plexus and meninges to dilute a circulating poison by an increased output of cerebrospinal fluid. Colorimetric comparison of suc-

Fig. 2.—Sections of the brain and of the cord of a dog lavaged with 0.1 per cent mercurochrome in physiologic solution of sodium chloride. The sections of the formaldehyde-hardened cord and brain are, beginning at the top, through the fourth ventricle, upper cervical, upper thoracic, lower thoracic, lumbar and lumbosacral cord. The diffuse and punctate hemorrhages involve mainly the white matter, being most marked in the cervical region and becoming less marked caudad and cephalad. The actual size is shown.

cessive specimens of lavage fluid collected at the needle of exit with the original solution introduced, indicates that the intrathecal concentration of mercurochrome increases as the lavage progresses. This may possibly be interpreted as a gradual failure, through exhaustion, of the choroid plexus and meninges to produce sufficient cerebrospinal fluid to dilute the mercurochrome, with the result that a lethal concentration of the drug in the subarachnoid space is attained.

METAPHEN

Toxicity.—There is no reference in the literature regarding the intrathecal toxicity of metaphen (4-nitro-3, 5 diacetoxy-mercuri-2-cresol). By intravenous injection, Raiziss and Severac [26] found that white rats tolerated the drug well in doses of 4 mg. per kilogram, and Birkhaug [25] reported that for rabbits doses of 3 mg. per kilogram, were well borne. Kolmer,[27] who used metaphen intrathecally in dogs, did not state how toxic it was by this route.

We used forty-one dogs in studying the effect of the intrathecal injection of metaphen. Twenty dogs were given the drug by the cisternal route, eleven by the spinal route and ten were lavaged with metaphen from the cisternal to the lumbar needle. In order to exclude as far as possible the factors of trauma and hemorrhage as causes of changes following the injection of metaphen, fifteen dogs in which punctures resulted in bloody fluids or in needle puncture trauma were omitted from the tables, and all brains and cords were examined for evidences of such trauma.

Table 3 is a summary of the results of the intracisternal injection of metaphen in fourteen dogs.

The maximum subtoxic dose was between 0.103 and 0.119 mg. per kilogram. The dogs receiving between 0.119 and 0.156 mg. per kilogram manifested toxic symptoms, but recovered, or apparently would have recovered if they had not been put to death. The one receiving 0.183 mg. per kilogram was killed when death impended thirteen days after the injection. One receiving 0.528 mg. per kilogram died on the third day. The minimum lethal dose of metaphen, when injected into the cistern, was therefore between 0.156 and 0.183 mg. per kilogram.

The animals that recovered manifested surprisingly few symptoms clinically to indicate that they had received a foreign substance intrathecally. Aside from a listlessness or inactivity that rarely amounted to stupor, anorexia and mild asthenia that lasted for one or two days, these dogs acted normally; no permanent after-effects were noted. In two dogs that received 0.095 and 0.119 mg. per kilogram, respectively, there occurred more severe reactions. They remained weak and ataxic up to the time that they were killed on the fourth day. In each of these dogs, however, cisternal hemorrhages were found post mortem that may have been of traumatic origin; but these dogs are included in the series because clear fluids were obtained at the time of puncture, and because it was doubtful whether the hemorrhages were the cause of the symptoms. Two dogs which had apparently fully recovered twenty-four hours after

26. Raiziss, G. W., and Severac, M.: J. Lab. & Clin. Med. **9**:71, 1923; J. Infect. Dis. **40**:447, 1927.

27. Kolmer (footnote 10, first reference).

TABLE 3.—*Summary of Experiments with Intracisternal Injection of Metaphen in Dogs*

Dog*	Weight of Dog, Kg.	Amount and Concentration of Solution Injected	Amount Metaphen Injected, Mg.	Amount Metaphen per Kg., Mg.	Results	Structural Changes
1	6.4	1 cc. of 1:10,000	0.1	0.016	No toxic symptoms; complete recovery; dog killed on 12th day	No changes
2	5.9	1 cc. of 1:10,000	0.1	0.017	No toxic symptoms; complete recovery; dog killed 16 days later	No changes
3	4.5	1.5 cc. of 1:10,000	0.15	0.033	During the injection dog stopped breathing, resuscitated; complete recovery; killed 1 mo. later	Fibrotic thickening of the meninges
4	7.3	1 cc. of 1:2,500	0.4	0.055	No toxic symptoms; complete recovery; dog killed 16 days later	Small epidural hemorrhage at level of cistern
5	6.8	0.5 cc. of 1:1,000	0.5	0.073	Weakness for 12 hr.; apparent recovery after 24 hr.; dog died during an attempted repuncture of cistern	Needle-track traumatic hemorrhage in medulla; subdural hemorrhage; serofibrinous leptomeningitis
6	7.8	1.5 cc. of 1:2,500	0.6	0.077	No toxic symptoms; complete recovery; dog killed on 3d day	Acute serofibrinous leptomeningitis
7	6.3	0.6 cc. of 1:1,000	0.6	0.095	Listlessness and surliness for 2 days; asthenia on 4th day; dog killed on 4th day	Traumatic hemorrhage in medulla and over base of brain; acute serofibrinous leptomeningitis
8	6.8	0.7 cc. of 1:1,000	0.7	0.103	No toxic symptoms; complete recovery; dog killed on 15th day	Edema and fibrotic thickening of the leptomeninges
9	12.7	1.4 cc. of 1:1,000	1.4	0.110	Dog walking about 24 hr. later; cistern repunctured and bloody fluid obtained; dog died the next day, apparently as result of second puncture	Subarachnoid hemorrhages over cord and base of brain; serofibrinous leptomeningitis
10	5.9	0.7 cc. of 1:1,000	0.7	0.119	Dog listless; able to walk on 2d day, but remained asthenic and ataxic, especially in hind legs; killed on 4th day	Fibrinopurulent leptomeningitis; small cisternal hemorrhage
11	7.7	1 cc. of 1:1,000	1.0	0.130	Dog apparently fully recovered when killed 24 hr. later	Fibrinopurulent leptomeningitis
12	6.4	1 cc. of 1:1,000	1.0	0.156	Dog apparently fully recovered when killed 24 hr. later	Acute serofibrinous leptomeningitis
13	5.5	1 cc. of 1:1,000	1.0	0.183	Dog stuporous for several days, finally lapsing into coma; killed when moribund on 13th day	Necrosis of small area in medulla; chronic healed fibrinous meningitis
14	7.6	4 cc. of 1:1,000	4.0	0.528	Dog lethargic; died on 3rd day	Acute serofibrinous meningitis

* Six dogs, all of whom had histologic evidence of an aseptic leptomeningitis, were omitted from this series because death was definitely due to trauma at first puncture.

the injection, and which were repunctured at that time to obtain cerebrospinal fluid for examination, died. They were included in the series because necropsy revealed extensive basal and cisternal hemorrhages in both animals and, in one also a fresh needle-track hemorrhage in the medulla, which we attributed to trauma incurred at the second puncture.

When injected into the spine, metaphen was less lethal than when injected into the cistern. In fact, a dose of 0.220 mg. per kilogram

TABLE 4.—*Summary of Experiments with Intraspinal Injection of Metaphen in Dogs*

Dog*	Weight of Dog, Kg.	Amount of Solution Injected	Amount Metaphen Injected, Mg.	Amount Metaphen per Kg. Mg.	Results	Structural Changes
1	6.6	1 cc. of 1:10,000	0.1	0.015	Complete recovery; no toxic symptoms; dog killed 8 wk. later	No changes
2	3.9	1 cc. of 1:10,000	0.1	0.026	Complete recovery; no toxic symptoms; dog killed 1 mo. later	No changes
3	6.8	2 cc. of 1:5,000	0.4	0.059	Complete recovery; dog killed 2 wk. later	Moderate hyperemia of cord, brain and leptomeninges
4	9.7	1.5 cc. of 1:2,500	0.6	0.062	Complete recovery; dog killed on 21st day	Early fibrinous leptomeningitis limited to cord region
5	6.8	1.5 cc. of 1:2,500	0.6	0.088	No toxic symptoms; complete recovery; dog died of pneumonia 9 days later	Edema of leptomeninges
6	8.2	2 cc. of 1:2,500	0.8	0.097	No toxic symptoms; dog apparently recovering; killed on 2d day	Edema of leptomeninges
7	8.7	3 cc. of 1:2,500	1.2	0.138	Complete recovery; no toxic symptoms; dog died 1 mo. later of pneumonia	Hyperemia of leptomeninges and cord
8	14.5	5 cc. of 1:2,500	2.0	0.138	Dog lethargic and died 10 hr. later	Fibrinopurulent leptomeningitis limited to cord region
9	6.8	1.5 cc. of 1:1,000	1.5	0.220	Dog listless for several days; complete recovery; killed on 21st day	No changes

* Two dogs were omitted from the series because both had traumatic transverse myelitis.

(table 4) failed to kill the dog into which it was injected intraspinally, whereas one of 0.183 mg. per kilogram killed the animal that received it in the cistern. One dog that received only 0.138 mg. per kilogram died ten hours after the injection, whereas two other dogs that received 0.138 and 0.220 mg. per kilogram, respectively, did not die. We believe that this apparent contradiction was due to the fact that the first dog, which succumbed, was one of our earlier animals in which the injection was made without proper control of the pressure. In view of our later knowledge that even small injections may enormously increase the intracranial pressure, we believe that death in this dog was attributable not to the toxic effect of metaphen, but to increased intracranial pressure.

In only one dog were there clinical symptoms of a severe reaction. This was one which was given the maximum dose of 0.220 mg. per kilogram, and which was stuporous for several days afterward, but which finally completely recovered, being killed three weeks later.

The maximum subtoxic dose of metaphen by intraspinal injection was therefore between 0.138 and 0.220 mg. per kilogram.

Lavage.—Ten dogs were lavaged from the cistern to the lumbar region with metaphen. Both a 1:10,000 and a 1:25,000 dilution of metaphen in physiologic solution of sodium chloride were used.

The average time of a lavage was between twenty and thirty minutes, during which from 30 to 40 cc. of fluid was allowed to run into the cistern, and about from 50 to 80 per cent of this amount of fluid was collected at the lumbar region. It took between one and a half and three minutes for the fluid introduced at the cistern to appear at the lumbar region. This determination was made by adding some methylene blue to the original solution and noting the first appearance of the colored fluid at the needle of exit.

Lavage is attended with many accidents to the dog incident to the movement of the dog or to that of the needle during the course of the experiment, so that five of these dogs died either during the lavage experiment or within the following thirty-six hours as a result of medullary or cisternal trauma. Of the five that recovered, one died two weeks later of a confluent bronchopneumonia, and four were put to death six weeks later after their complete recovery. The symptoms manifested by the animals that recovered were generally the same as those following the simple injection of metaphen, i. e., listlessness and irritability, which generally disappeared in a few days. Although one dog that recovered was lavaged with a 1:10,000 solution, we are not certain that such a concentration is harmless when large amounts are employed. However, cistern-lumbar lavage with a 1:25,000 solution of metaphen was found to be well tolerated.

Bactericidal Activity.—Metaphen (4-nitro-3, 5-diacetoxymercuri-2 cresol) was introduced by Raiziss and Severac [26] as an antiseptic with a high bactericidal coefficient and a low toxicity. These observers found that in twenty-four hours it inhibited the growth of S. aureus in dilutions up to 1:20,400,000. In one minute exposures in a 50 per cent serum, metaphen destroyed this organism in a 1:10,000 dilution, being ten times more powerful than hexylresorcinol S. T. 37 and twenty-five times more powerful than mercurochrome under the same conditions.

Birkhaug,[25] comparing various germicides for their bactericidal activity against several organisms, obtained similar results. Compared with phenol as having a bactericidal coefficient of 1 for B. coli after forty-eight hours' incubation, he found that metaphen had a phenol coeffi-

cient of 250, hexylresorcinol one of 50, mercuric chloride one of 125, tincture of iodine one of 38 and mercurochrome one of 2. He found the highest dilution of metaphen in distilled water capable of killing B. coli, the most resistant of all organisms that he tried, after ten, but not after five, minutes' contact, to be 1 : 20,000, compared with 1 : 160 for mercurochrome.

When diluted in equal parts of blood serum and distilled water, all of the antiseptics showed markedly reduced antiseptic power. Under these conditions, Birkhaug found that metaphen was still a more effective bactericide than any of the other antiseptics that he used, being over sixty times more powerful than mercurochrome.

Thalhimer [24] found that for exposures of two and one-half minutes metaphen was bactericidal to a twenty-four hour broth culture of B. typhosus in dilutions up to 1 : 50,000. For fifteen minute exposures, he found it to be bactericidal in dilutions up to 1 : 100,000.

Since the presence of blood serum, according to Birkhaug, diminished the bactericidal power of metaphen to one tenth of its value when it was diluted in distilled water, we first determined whether cerebrospinal fluid had such an inhibitory effect.

We found that metaphen when diluted either in physiologic solution of sodium chloride or in equal parts of cerebrospinal fluid and physiologic solution of sodium chloride was bactericidal to twenty-four hour broth cultures of B. coli in five minute exposures in dilutions of 1 : 50,000 and in fifteen minute exposures in dilutions of 1 : 100,000. The average phenol coefficient of metaphen diluted in physiologic solution of sodium chloride was 642, the same as its phenol coefficient when it was diluted in 50 per cent cerebrospinal fluid. It is therefore apparent that normal cerebrospinal fluid has practically no effect on the bactericidal activity of metaphen. As compared with mercurochrome, which we found to be bactericidal in 1 : 1,000 dilution, under similar conditions, metaphen was found to be over 50 times more bactericidal.

CEREBROSPINAL FLUID CHANGES

The intrathecal injection of practically any substance is followed by changes in the cerebrospinal fluid. These changes were studied in twenty-eight dogs, twenty after the injection of metaphen and eight after the injection of mercurochrome.

Within one hour after the injection of the chemical, provided the dog did not die before this time, the cerebrospinal fluid manifested changes that persisted for from three to seven days, depending on the severity of the reaction. The fluid was tinged fluorescent pink for several hours after the injection of mercurochrome or yellow after the injection of metaphen. It was usually clear, but at times was turbid. The cerebrospinal fluid pressure was increased, except in those cases in which the

fluid was too thick and gelatinous to flow freely through the needle; in these cases, the pressure was decreased. Within twelve hours there occurred an increase in the protein content of the fluid, at times slight and at other times so great that the fluid coagulated almost immediately after withdrawal from the body. This increase in protein was practically all albumin, there being hardly any increase in globulin. The coagulum that formed was composed of fibrin, and contained many polymorphonuclear leukocytes, lymphocytes and erythrocytes.

The cell content of the fluid was increased. There was usually an exudation of erythrocytes first, as observed by Kolmer [18] following intrathecal injections of mercurochrome, up to several thousand per cubic millimeter. This initial exudation of erythrocytes probably accounts for the yellow color and positive benzidene reaction of the fluid obtained at subsequent punctures. The white blood cells were also increased in number and were both of the polymorphonuclear and lymphocytic type, the former predominating. There were frequently over 1,000 cells per cubic millimeter. In two cases, after injection of metaphen, many so-called endothelial cells were present also. In four instances in which metaphen was administered, and in which the cerebrospinal fluid contained an increase in protein so marked that the fluid coagulated within a few minutes after its withdrawal, the cell counts were relatively low, being 4, 13, 15 and 75, respectively. This phenomenon of albuminocytologic dissociation we have been unable to explain. It may be due to the same cryptogenic mechanism that is responsible for the similar changes occurring in the cerebrospinal fluid in the Froin syndrome of spinal subarachnoid block.

Cultures and smears made from the cerebrospinal fluid were repeatedly negative for organisms, indicating that the changes in the fluid were due to an aseptic meningitis. The cerebrospinal fluid changes enumerated occurred even when as little as 0.015 mg. of metaphen per kilogram (the lowest dose that we used) was injected intrathecally.

PATHOLOGY

The brains and cords were fixed in 4 per cent formaldehyde and histologic sections were stained with hematoxylin-eosin and sudan III, and by the method of Pal-Weigert. In addition, bacterial stains were made when indicated.

Following the injection of mercurochrome or of metaphen, as following the injection of practically any substance into the subarachnoid space, there occurred a meningeal reaction.

This reaction was observed to vary from simple hyperemia and edema of the meninges to serofibrinopurulent aseptic leptomeningitis, depending on the amount of the drug injected, the individual susceptibility of the animal to the drug and the length of time elapsed between the injection and the necropsy.

Grossly, the cords and brains showed scant changes, even when the clinical symptoms were severe. At times there was a small localized subdural hemorrhage at the site of puncture, and in the more severe meningeal reactions perivascular white streaks in the sulci were visible, especially over the base of the brain. Frequently there was hyperemia of the blood vessels of the brain and cord.

After the injection of mercurochrome, the distribution of the dye over the cord and the brain varied with the site of the injection and the

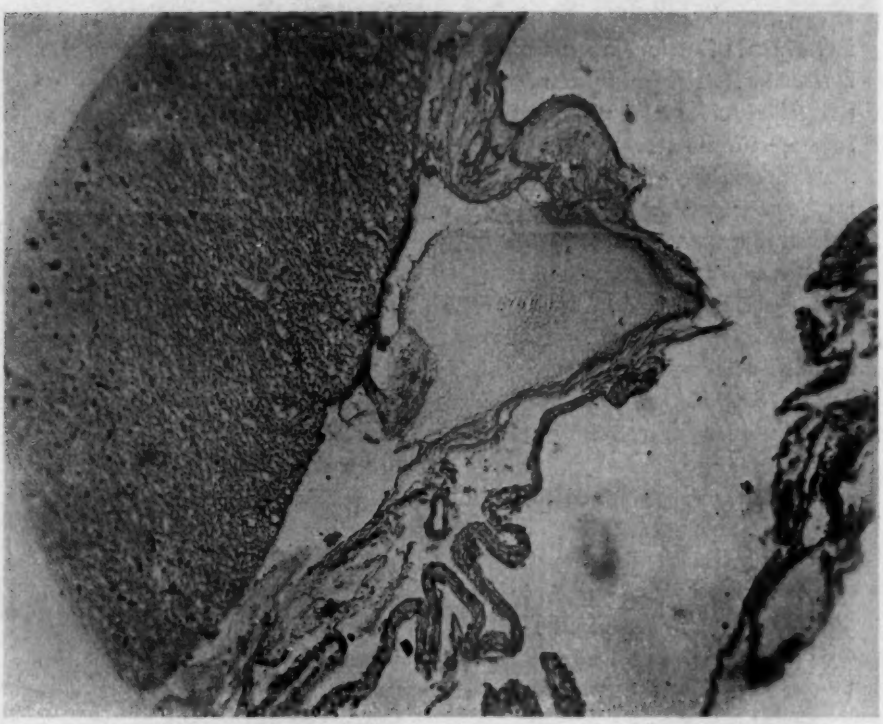

Fig. 3.—Edema of the spinal subarachnoid space eleven days after intraspinal injection of mercurochrome. There is practically no cellular reaction; hematoxylin and eosin; × about 120.

amount injected. In general, it was found over the cord, the medulla and the base of the brain. When the injection was made into the cistern, the dye had a wider distribution over the base of the brain and the cortex than when injected into the spine. Sometimes, with larger amounts, the stain partially covered the cortex. Only when very large quantities were injected, however, were the lateral ventricles stained. The order of frequency of distribution of the dye when injected intrathecally was, cord, medulla and pons, base of brain, cortex and finally ventricles. The

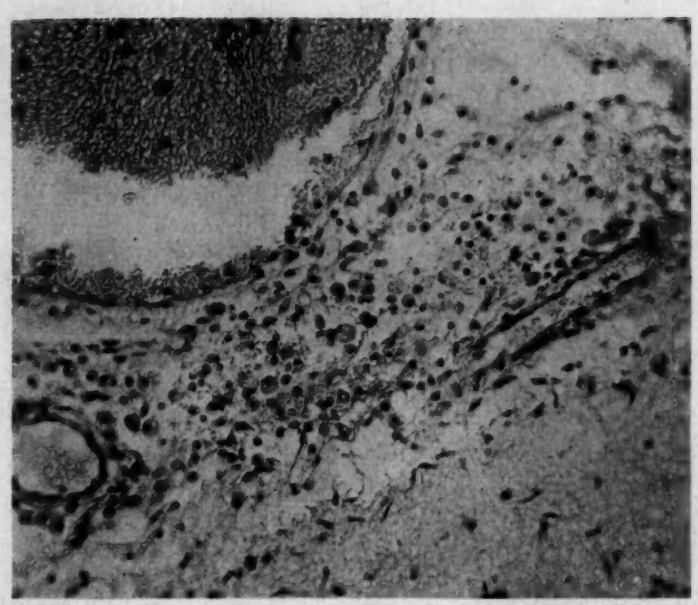

Fig. 4.—Acute aseptic serous meningitis five days after injection of mercurochrome. There are many endothelial and relatively few polymorphonuclear cells present in the subarachnoid space; hematoxylin and eosin; × about 200.

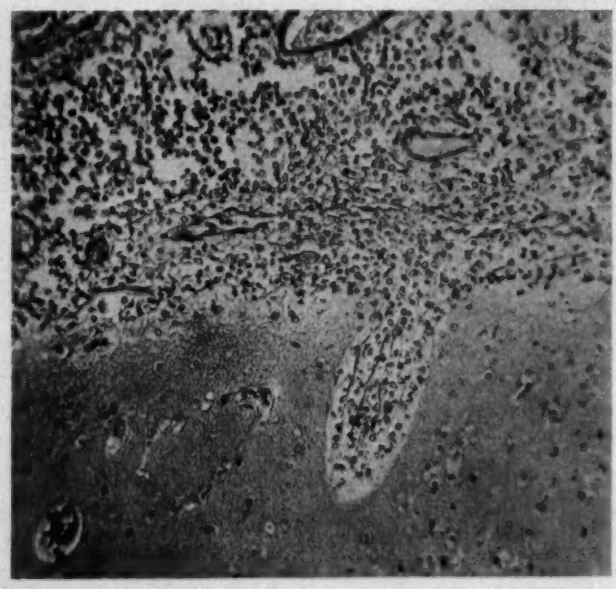

Fig. 5.—Purulent exudate in the leptomeninges twenty-four hours after injection of mercurochrome; hematoxylin and eosin; × about 120.

stain did not appear to penetrate below the pia mater and disappeared within a few days.

Histologically, however, there were evidences of the presence of an aseptic meningeal reaction even in animals receiving minimal doses of the drugs, the cords and brains of which appeared grossly normal. The changes were essentially the same regardless of the drug used.

Early there occurred a hyperemia with some diapedesis of erythrocytes into the subarachnoid space. The spaces were distended by edema or by fibrinous exudate (fig. 3). There was usually an increased

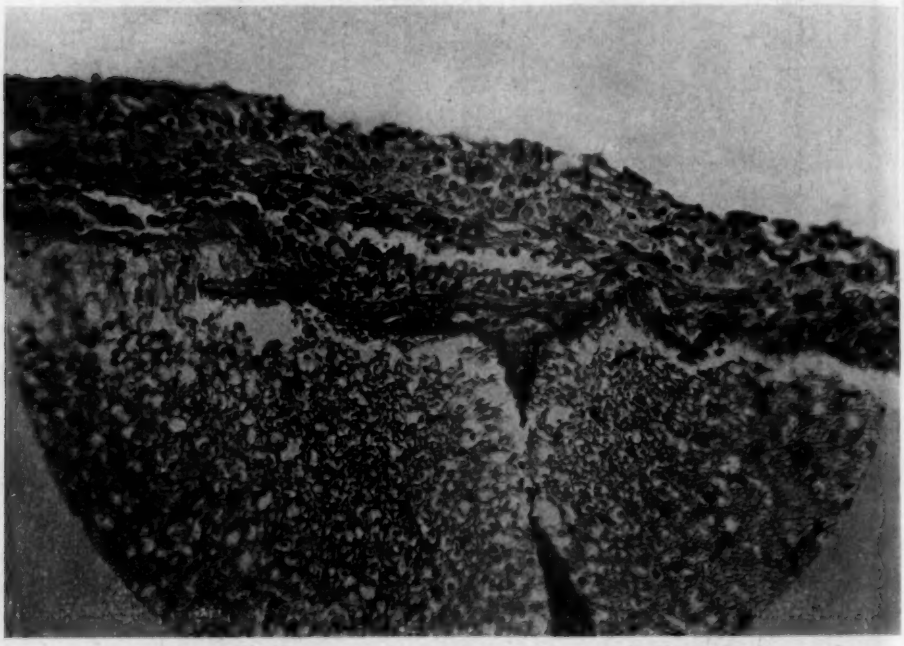

Fig. 6.—Organizing fibrinopurulent meningitis eleven days after intraspinal injection of mercurochrome. The predominating cells are fibroblasts, polymorphonuclear leukocytes and endothelial cells; hematoxylin and eosin; × about 180.

number of polymorphonuclear leukocytes, lymphocytes and endothelial cells (fig. 4). In the more severe reactions there was a frankly purulent exudate in the subarachnoid space (fig. 5). If the dogs were killed from five to six days after the injection, there were usually many fibroblasts and plasma cells present in the leptomeninges (fig. 6). After several weeks, all that was left to indicate that a meningeal reaction had occurred was a fibrotic thickening of the meninges. Frequently, if the dog was killed from two to three weeks after the injection, no residual pathologic changes were discernible.

In those dogs in which transverse myelitis occurred, there were degeneration of the white matter as seen in Pal-Weigert stains and replacement of the gray matter by débris, granule cells and glial cells (figs. 7 and 8). In one dog in which clinical transverse myelitis developed, the symptoms were explained on the basis of a localized fibrinous

Fig. 7.—Necrosis of the gray matter of the cord eleven days after intraspinal injection of mercurochrome. The normal tissue is replaced by granule cells and glial cells; hematoxylin and eosin; × about 200.

meningitis of the cord. Except for hyperemia, there were no other changes in the parenchyma of the cord and brain. Bacterial stains revealed no organisms. Except for an occasional small fat droplet in the meninges and about the central canal, sudan III-stained sections also showed no morbid changes.

In dogs that received lethal doses of mercurochrome into the cistern, except for the stained leptomeninges, the histologic conditions were entirely normal. This may have been because death occurred before detectable changes had time to occur.

As described, dogs in which lavage with a 0.1 per cent mercurochrome was performed frequently had, in addition to the meningeal reaction, changes in the cord and brain stem consisting of disseminated areas of hemorrhage and necrosis.

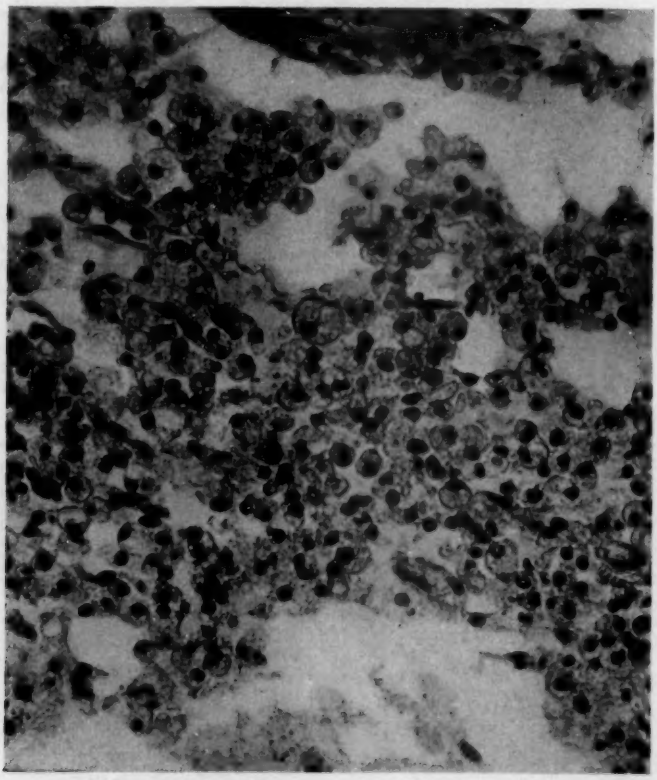

Fig. 8.—Higher magnification of granule cells in necrotic foci. These are large, pale cells with a latticed cytoplasm and with an eccentrically placed oval nucleus; hematoxylin and eosin; × about 350.

COMMENT

In our search for a powerful antiseptic with a low intrathecal toxicity, we studied the effect of the intrathecal injection of mercurochrome and of metaphen. It became apparent early in our work that the strength of the chemical was not so much a factor as the dosage in milligrams per kilogram of body weight. Thus, dogs that were treated by injection of 1:1,000 solution of metaphen did not show any more severe symptoms

than those that were treated by injection of a 1:10,000 solution, provided the dose in milligrams per kilogram was approximately the same.

The dosages in milligrams per kilogram are given in table 5, which summarizes the results that we obtained with these chemicals. Equivalent dosages are also given for an adult human being weighing 60 kilograms (132 pounds). It is evident that both these drugs have a high intrathecal toxicity, both being somewhat less toxic when injected into the spinal subarachnoid space than when injected into the cistern. Of the two, metaphen is the less toxic and less lethal. Not only are its intracisternal subtoxic and sublethal doses less than those of mercurochrome, but the margin of safety between the subtoxic and the lethal dose is greater for metaphen. Morevover, we observed that the dogs receiving

TABLE 5.—*Comparison of Maximum Subtoxic and Minimum Lethal Doses of Mercurochrome and Metaphen*

Method	Maximum Subtoxic Dose		Minimal Lethal Dose	
	Mercurochrome	Metaphen	Mercurochrome	Metaphen
Intracisternal injection				
Mg. per Kg. body weight.....	0.07–0.11	0.10–0.12	0.11–0.15	0.16–0.18
Cc. of 1:1,000 solution for 60 Kg. (132 lb.) adult human being.....	4.0 –6.6	6.0 –7.2	6.6 –8.7	9.6 –10.8
Intraspinal injection				
Mg. per Kg. body weight.....	0.17–0.22	0.14–0.22	(Highest dose used, 1.21 Mg. per Kg., not fatal)	(Highest dose used, 0.22 Mg. per Kg., not fatal)
Cc. of 1:1,000 solution for 60 Kg. (132 lb.) adult human being.....	9.9 –13.2	8.3 –13.2		

lethal doses of metaphen intracisternally, usually died from one to thirteen days after the injection, apparently as a result of the severe aseptic meningitis. However, those that succumbed to the injection of mercurochrome into the cistern usually died a respiratory death, within thirty minutes after the injection, indicating that there was a direct action of the drug on the vital medullary centers. The fact that the medullary nuclei appeared normal on histologic examination does not disprove this theory.

Intraspinally, practically the same subtoxic doses were found for both drugs. Minimum lethal doses by this route were not given, because the animals survived the highest intraspinal doses that we gave. However, mercurochrome is a more dangerous drug to use intraspinally, for myelomalacia was much more common, following the intraspinal injection of mercurochrome than following that of metaphen.

In making up our dilutions of mercurochrome and metaphen for lavage, physiologic solution of sodium chloride was used. The question naturally arises as to what part the salt solution played in the production of the clinical and morbid changes, for it has been shown by Weed

and Wegeforth [28] that physiologic solution of sodium chloride itself is harmful when injected intrathecally. In view of the fact that none of a preliminary series of dogs in which we performed lavage with greater amounts of physiologic solution of sodium chloride showed nearly so severe reactions as those manifested by the dogs lavaged with either mercurochrome or metaphen, we feel convinced that these chemicals and not the salt solution played the major rôle in producing the changes observed.

The aseptic meningeal reaction that follows the intrathecal injection of metaphen or mercurochrome is probably the result of an injury to the meninges causing increased permeability of the blood vessels with exudation of erythrocytes and plasma protein, i. e., mainly albumin, into the subarachnoid space. Whether or not such a reaction in the presence of bacterial meningitis would be beneficial per se as a result of the outpouring of immune bodies along with the plasma proteins from the blood into the cerebrospinal space, which is usually devoid of such immune bodies, or as a result of a nonspecific protein reaction is open to question.

In the clinical use of antiseptics in suppurative meningitis, much more can be expected from lavage with the antiseptic than from its simple injection. The ideal method of reaching most of the subarachnoid space is by lavage from two needles inserted through trephined openings over the right and left sides of the vertex to a cisternal or lumbar needle, as has been done by Wegeforth and Essick,[9] Kolmer [27] and Stewart [11] in their experimental work on meningitis. We, however, have limited ourselves to lavage from the cistern to the lumbar region, as we believe this to be the route of choice in the lavage treatment for meningitis in man. We are now trying out lavage from the lumbar region to the cistern in the hope that it will be accompanied with fewer accidental deaths than cistern-lumbar lavage and also because, as suggested by Pollock and Favill,[29] it may be a better method of reaching the cortical subarachnoid space than by cistern-lumbar lavage.

Because mercurochrome in bactericidal concentrations is lethal, its employment as an intrathecal disinfectant is contraindicated. Its "noninjurious affinity for body tissues" does not apply to the tissues comprising the central nervous system. In view of the fact, however, that metaphen is from 25 to 100 times more bactericidal than mercurochrome, we have not discarded it, as we have mercurochrome. As far as we know, metaphen has not been used previously to any extent in the treatment for meningitis. Kolmer [27] mentioned having used it in this disease

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28. Weed, L. H., and Wegeforth, P.: J. Pharmacol. & Exper. Therap. **13**:317, 1919.

29. Pollock, L. J., and Favill, J.: The Human Cerebrospinal Fluid, New York, Paul Hoeber, 1924, p. 475.

with disappointing results, but he stated that his experience with it had been limited.[30] We believe that it is worthy of further trial in meningitis, for it can be introduced in bactericidal strength in subtoxic doses.

SUMMARY

Subthecal punctures and injections in the dog are attended by difficulties and frequently by injuries to the animal. With the proper technic, however, these may usually be circumvented.

The maximum subtoxic dose of mercurochrome by intracisternal injection was between 0.07 and 0.11 mg. per kilogram; the minimum lethal dose was between 0.11 and 0.15 mg. per kilogram. By intraspinal injection, the toxicity was less, the maximum subtoxic dose being between 0.17 and 0.22 mg. per kilogram.

The maximum subtoxic dose of metaphen by intracisternal injection was between 0.10 and 0.12 mg. per kilogram; the minimum lethal dose was between 0.16 and 0.18 mg. per kilogram. By intraspinal injection, the toxicity was less, the maximum subtoxic dose being between 0.14 and 0.22 mg. per kilogram.

Lavage from the cistern to the lumbar region with a 1:1,000 solution of mercurochrome was fatal to dogs, but lavage with a 1:25,000 solution of metaphen was well borne.

Mercurochrome was bactericidal in dilutions up to 1:1,000 under the same conditions in which metaphen was bactericidal in dilutions up to 1:50,000.

After the intrathecal injection of either mercurochrome or metaphen, the following changes occurred in the cerebrospinal fluid: (*a*) increased pressure; (*b*) increased number of cells, first of erythrocytes and later of polymorphonuclear leukocytes and lymphocytes, the former predominating; (*c*) increased protein content, mainly due to albumin to such an extent that the fluid coagulated on standing.

An aseptic serofibrinopurulent leptomeningitis followed the intrathecal injection of either mercurochrome or metaphen.

Mercurochrome should not be administered intrathecally clinically, because sublethal doses are not bactericidal.

Metaphen deserves further investigation as an intrathecal disinfectant, for not only does it have a larger margin of safety than mercurochrome, but it is bactericidal in sublethal doses.

30. Kolmer, J. A.: Personal communication to the authors.

CONGENITAL ATRESIA OF THE ESOPHAGUS WITH TRACHEO-ESOPHAGEAL FISTULA

REPORT OF EIGHT CASES *

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According to Mackenzie,[1] the first recorded case of congenital atresia of the esophagus was that of Durston in 1670. In 1861, Hirschsprung [2] collected the reports of 10 cases from the literature and added 4 of his own. In 1884, Mackenzie [1] collected the reports of 62 cases and reported a new case. In 43 of the cases recorded to that time a tracheo-esophageal fistula was present. The most complete review of the literature was that of Plass,[3] who in 1919 collected the reports of 136 cases of esophageal atresia. A careful search of the literature has revealed 85 additional cases of esophageal atresia reported since Plass' review; 61 of these were associated with tracheo-esophageal fistula, and 14 were not, as shown by postmortem examination. Since an autopsy was not permitted in 10 cases, the presence or absence of a fistula was not fully established in all of these cases, although the clinical findings pointed to its presence in the majority of these instances. The presence of the atresia, however, was definitely diagnosed by the characteristic clinical picture. Thus, to date, including the 8 cases described here, there are 255 recorded cases of congenital esophageal atresia, in 205 of which a tracheo-esophageal fistula was present, and in 40 of which it was absent.

The aforementioned number of cases cannot, of course, be taken as the number of cases that have occurred. Authors are at great variance in their conceptions of the frequency of this condition. As stated in an accompanying clinical report, the indications are that many more cases have occurred than have been reported or even diagnosed.

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* From the Division of Laboratories, Mount Sinai Hospital, New York, and the Carnegie Institution of Washington, Department of Embryology, Baltimore.

1. Mackenzie, Morell: A Manual of Diseases of the Throat and Nose, New York, William Wood & Company, 1884, vol. 2, p. 149.

2. Hirschsprung, cited by Mackenzie (footnote 1).

3. Plass, E. D.: Congenital Atresia of the Esophagus with Tracheo-Esophageal Fistula, Johns Hopkins Hosp. Rep. **18**:259, 1919.

EIGHT CASES

The symptoms and the clinical aspects of this anomaly in 8 cases may be found in the accompanying report. A brief summary of the characteristic symptoms is as follows:

The infant is usually well nourished and well developed at birth, and with the exception of the presence of a large amount of mucus in the mouth and pharynx and often in the trachea, no abnormalities are suspected. Occasionally this may cause asphyxia. The most characteristic and constant symptom is the typical episode that attends each attempt at feeding. The infant accepts fluids readily enough, but after a few swallows stops, ceases to breathe, becomes cyanotic and regurgitates a frothy discharge through the nose and mouth. A period of almost lifeless relaxation may follow this episode, but the child almost always recovers and repeats the performance, if given the opportunity. The weight falls steadily, and the infant rapidly becomes dehydrated. The average loss of weight before death is from 25 to 40 per cent. Physical examination a short time after birth may reveal little except a whitish, frothy mucoid material in the nasopharynx. Depression of the anterior fontanel and a dry, adherent skin may be seen later as dehydration becomes marked. The epigastrium is often distended, and percussion may reveal tympany of the stomach, a sign of the presence of the tracheo-esophageal fistula. For a few days the stools are normal in quantity and are typical meconium. Later they become scant or absent and are composed merely of bile-stained mucus—the stools that characterize starvation. The urine is scanty and concentrated, and early suppression is seen. The temperature is usually raised by the advent of inanition fever after from two to three days, and may be influenced by the bronchitis and bronchopneumonia that commonly complicate the clinical picture. The temperature may, however, remain normal or become subnormal. The diagnosis is confirmed by the passage of a catheter, which meets an obstruction at about from 10 to 12 cm. from the alveolar ridges. Roentgen examination after the introduction of a barium mixture into the pharynx reveals a dilated culdesac reaching usually to about the fourth thoracic vertebra.

CASE 1.—B. D., a boy, died four days after birth. Postmortem examination revealed a poorly nourished infant on whom a gastrostomy had been performed. The pharynx led into a widely dilated esophagus, which was completely closed at a point 1 cm. above the bifurcation of the trachea. From this point on, the esophagus was nonexistent as a patent tube, but continued as a ridge in the form of a longitudinal thickening on the posterior wall of the trachea. It continued in this fashion to a point a little above the bifurcation of the trachea, where it once more emerged as a patent tube. Its lumen at this point communicated with the trachea in a manner resembling the entrance of the ureter into the bladder. It joined the stomach in a normal fashion. A widely patent ductus arteriosus was present, as well as a patent foramen ovale (fig. 1 *A* and *B*).

Case 2.—B. H., a boy, died six days after birth. Postmortem examination revealed a fairly well developed and well nourished infant. The upper third of the esophagus terminated in a short fibrous cord which was firmly attached to the posterior wall of the trachea near the bifurcation. The lower two thirds of the esophagus was found to open into the lumen of the trachea just below this point and above the bifurcation. The heart was enlarged, owing to a dilatation of the right auricle and right ventricle. A widely patent ductus arteriosus was present. The foramen ovale was closed. Aspiration bronchopneumonia and malposition of the transverse colon were also present.

Case 3.—K. I., a boy, died six days after birth. Postmortem examination revealed the upper part of the esophagus to be dilated and to end as a blind pouch about 4 cm. below the hyoid bone. It was markedly hypertrophied. No cord was seen extending from this blind end caudadly. The trachea at its bifurcation was

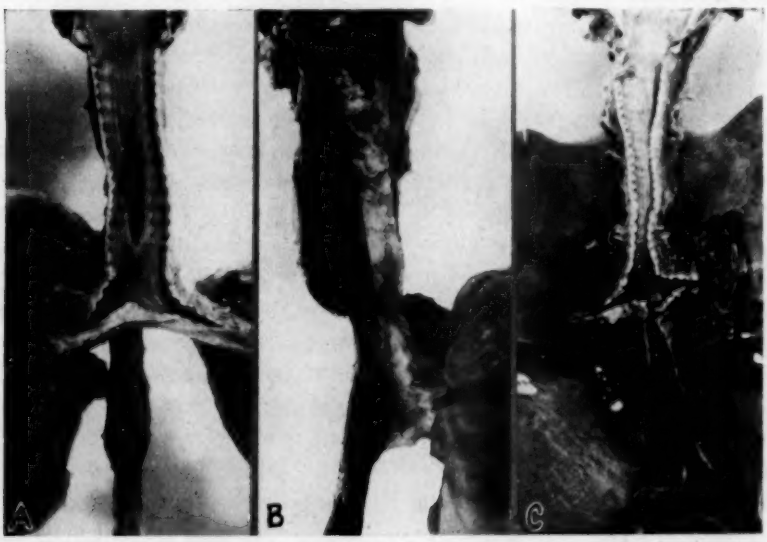

Fig. 1.—Specimens showing congenital atresia of the esophagus with tracheoesophageal fistula: *A* and *B*, case 1; *C*, case 3.

seen to divide into three divisions, two lateral cartilaginous ones, which were evidently the bronchi, and a mesial noncartilaginous one, which was the lower end of the esophagus and extended to the stomach. The stomach was small and filled only with a mucoid secretion. No other abnormality was present in the gastrointestinal tract. The heart was entirely normal, except for a huge patent foramen ovale, measuring 1.5 cm. in diameter. It appeared almost like a complete interauricular septal defect. Bronchopneumonia was present (fig. 1 *C*).

Case 4.—B. W. died five days after birth. Postmortem examination revealed the upper portion of the esophagus ending as a blind pouch about 4 cm. long. This blind end was still attached to the lower portion of the esophagus by a very fine film of tissue. The lower portion of the esophagus entered the trachea about 2 cm. above the bifurcation. A patent foramen ovale was present, and the lungs were partially atelectatic.

CASE 5.—H. G., a boy, died seven days after birth. Postmortem examination revealed the upper third of the esophagus ending in a culdesac which was attached by a thin string of fibrous tissue to the posterior peritracheal tissue. The lower two thirds of the esophagus was in direct communication with the trachea just at its bifurcation. The other pathologic findings included a patent foramen ovale and bronchopneumonia.

Drs. Lederer and Wilson of the Jewish Hospital of Brooklyn permitted us to study and report the following 3 cases.

CASE 6.—B. W., a boy, died nine days after birth. Postmortem examination revealed the upper part of the esophagus ending in a blind sac about 6 mm. in diameter and 5 cm. in length. For a distance of 1.5 cm., it extended as a cordlike structure, which became continuous with the distal 6 cm. of the lower part of the esophagus. The latter was of normal caliber and structure and communicated with the trachea by a slitlike fistulous connection, situated about 0.5 cm. above its bifurcation. The foramen ovale was slightly patent, and the lungs were somewhat atelectatic.

CASE 7.—B. K., a boy, died six days after birth. Postmortem examination revealed the upper portion of the esophagus as a dilated, hypertrophied sac about 4 cm. in length. It continued as a cord along the posterior aspect of the trachea to about 1 cm. above its bifurcation, where it again became patent, communicated with the trachea and then continued normally to the stomach. The other abnormal findings included the absence of the left kidney and ureter, a patent foramen ovale and patent ductus arteriosus and bronchopneumonia.

CASE 8.—B. L., a boy, died eight days after birth. Postmortem examination revealed the upper fourth of the esophagus ending blindly as a dilated pouch. Its lumen was twice that of the lower part of the esophagus. The two portions were separated by a distance of 1 cm., and no connecting cord was present. The fistulous opening was just above the bifurcation. The skin was dry and wrinkled. The foramen ovale was closed, but the ductus arteriosus was patent. Bronchopneumonia was present.

A summary of the histology of the first 4 cases is presented later.

GROSS PATHOLOGY

As has been pointed out several times, the gross picture of the anomaly is markedly constant. Reference to the appended photographs of two of the specimens demonstrates this striking change clearly. The upper portion of the esophagus begins normally, but ends as a blind sac at varying distances below the larynx. Fischer (Henke and Lubarsch [4]) stated this length to be between 1.5 and 2 cm. Plass [3] gave the average length as about 3.4 cm. on the basis of 72 cases in 5 of which it measured from 1 to 1.9 cm. and in 9, 5 cm. or over, with the measurements of the others ranging between. The average length in the cases reported here agrees with that of Plass.[3] The pouch is almost always hypertrophied and uniformly dilated to a diameter of

4. Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 1, p. 84.

about 1 cm. or more, whereas the normal width of the esophagus in the new-born infant is about from 4 to 6 mm. This is true of practically all of the specimens studied here. Many believe the hypertrophy and dilatation to be the result of ineffectual attempts of the fetus to swallow amniotic fluid. Ballantyne,[5] Brigham,[6] Meyer[7] and Scheurer[8] noted the association of hydramnion with atresia of the esophagus and believed the hydramnion to be the result of the inability of the fetus to swallow and absorb the amniotic fluid. Plass[3] noted the association in 9 of 136 cases, but believed it to be mere coincidence. Others believe the hypertrophy to be due to an abnormal growth stimulus. Giffhorn[9] stated that since the posterior wall of the esophagus is fixed while growth continues, the entire blind sac must be pulled out in the manner that a traction diverticulum is formed, resulting in hypertrophy and dilatation.

The lower portion of the esophagus makes a fistulous connection with the trachea at a varying distance above the bifurcation of the latter into the bronchi, the average distance being about 0.5 cm.; in 2 of the cases reported here the fistula was at the bifurcation; in 1 case, just above the bifurcation; in 2 cases, 0.5 cm. above, and in 3, 1.0, 1.5 and 2 cm. above, respectively. Of 96 cases cited by Plass,[3] 94 showed the distance to be within this same range; in the remaining 2, a fistulous communication with a bronchus was found. A review of the macroscopic description in about 80 cases reported since Plass' review has shown that in these cases this distance fell within the same range. In 5 cases, a communication with a bronchus was noted; in 4 cases (Baranger,[10] Ellerbroek[11] and Hamm et al.[12]) the right bronchus was concerned, and in 1 the left bronchus. In the last case no actual fistula was present, and the esophagus continued as a cord. Sencert[13] stated that when the fistula is above the bifurcation, there are rarely concomi-

5. Ballantyne, J. W.: Manual of Antenatal Pathology and Hygiene, Edinburgh, W. Green & Sons, 1904, p. 462.

6. Brigham, R. O.: Atresia of the Esophagus, Am. Med. **35**:297, 1929.

7. Meyer, H.: Esophagusatresie und Hydramnion, Zentralbl. f. Gynäk. **53**: 1562, 1929.

8. Scheurer, D.: Ein Fall von kongenitaler Oesophagusatresie mit Oesophago-Trachealfistel, Schweiz. med. Wchnschr. **9**:1079, 1928.

9. Giffhorn, H.: Beitrag zur Atiologie der kongenitalen Atresia des Oesophagus mit Oesophagotrachealfistel, Virchows Arch. f. path. Anat. **192**:112, 1908.

10. Baranger, A.: Curieuses malformations des voies digestives supérieures, Arch. internat. de laryng. **34**:1077, 1928.

11. Ellerbroek, N.: Ein Beitrag zur Oesophagusatresia, München. med. Wchnschr. **69**:591, 1922.

12. Hamm, A., et al.: Un cas d'atrésie congénitale de l'oesophage, Bull. et mêm. Soc. anat. de Paris **94**:631, 1924.

13. Sencert, cited by Rogier, P.: Quelques considérations sur deux nouveaux cas d'imperforation de l'oesophage, Thèse de Paris, 1927, vol. 59.

tant malformations, while when it is at the same height or in the right bronchus, malformations of the heart and great blood vessels are the rule. No evidence of such an association has been encountered in a review of the literature. The fistulous opening has a characteristic crescent shape and has been aptly described as resembling the opening of the ureter into the bladder. This tracheal opening is always smooth, the mucous membranes of the tubes being continuous. The muscular walls frequently blend without any demarcation. Occasionally a groove-like depression may be seen running up the posterior membranous portion of the trachea from the region of the fistula. The lower portion of the esophagus seems to spring as a direct continuation from the muscular posterior wall of the trachea, beginning as a narrow tube measuring about from 4 to 7 cm. in length and gradually increasing in size to its normal caliber. It opens into the stomach in a normal fashion. In a case presented here, the lower portion of the esophagus measured 3 cm. in diameter at the fistulous end and 6 mm. at the cardiac end, and in another it measured 4 mm. at the fistulous end and 7 mm. at the cardiac end. According to Plass,[3] rudimentary tracheal cartilages have been found in the fistulous portion of the esophagus.

Occasionally, an overlapping of the two segments has been noted. In twelve of the fourteen specimens examined by Keith,[14] who collected them from medical schools and hospitals in England, the fundus of the upper segment overlapped the origin of the lower segment, and there was usually a greater or lesser degree of union between the musculature of the two portions of the esophagus and that of the trachea. An overlapping was noted in two of the specimens presented here; in one it was slight, and in the other marked. In the latter, the distance of overlapping was 1.7 cm. Keene[15] reported a similar case. If one does not accept mechanical factors as playing an important rôle in an elongation of the upper culdesac, this type of variation speaks against the theories concerning the development of the anomaly which have to do with pressure atrophy. Usually, however, the two segments of the esophagus are separated by a definite distance of about 1 cm. The maximal distance is about from 4 to 5 cm. In a case reported by Levys[16] the defect was 5 cm. The separation is usually bridged by a fibromuscular cord. No cord was present in two of the specimens presented here, and in one existed only as a fine film of tissue. Fischer (Henke and Lubarsch[4]) stated that a cord was present in 53 of 73 cases in which an exact study was made.

14. Keith, Arthur: Constrictions and Occlusions of the Alimentary Tract of Congenital or Obscure Origin, Brit. M. J. **1**:301, 1910.

15. Keene, R.: A Case of Irregular Separation of the Trachea from the Esophagus, St. Barth. Hosp. J. **28**:155, 1920-1921.

16. Cited in Henke and Lubarsch (footnote 4).

The question of the occurrence and type of congenital anomalies associated with this condition is of interest and importance. Several authors have stated that the frequent association of other anomalies incompatible with life makes surgical intervention for the atresia of the esophagus inadvisable. On the basis of cases recorded to date, this is not true and is an obstacle to advance in the treatment of infants with this condition. Reynolds and Morrison [17] stated that associated malformations are uncommon. The careful review of Plass,[3] however, shows that they are somewhat frequently present. Thus they were found in 59 of 94 cases in which the presence or absence of other anomalies was noted (patent ductus arteriosus and foramen ovale were not classed as anomalies), but few of the associated malformations were in themselves incompatible with life. By far the most common was atresia ani, noted in 24 cases. In a small proportion of the cases recorded since Plass' time, associated anomalies were noted, only one of which would have been fatal. The anomalies were: atresia duodeni, deformities of the hand, absence of the left kidney, double uterus and harelip. In 3 of the 8 cases reported here, other, relatively harmless abnormalities were present. They were absence of the left kidney, malposition of the transverse colon and slight deformity of the hand. The marked frequency of the presence of patent foramen ovale and a patent ductus arteriosus was noted. Every specimen presented at least one, and three had them both combined.

A presentation of the gross pathology would not be complete without reference to the frequent pulmonary complications seen with this anomaly. With the tracheo-esophageal fistula and the rapid inanition that sets in as a result of the atresia of the esophagus, it can be readily understood why tracheobronchitis and bronchopneumonia so commonly hasten the death of the infant. Thus aspiration bronchopneumonia was present in 5 cases and atelectasis in 2.

HISTOLOGY

As a general rule, gross specimens have been kept on exhibition. One is reluctant to section specimens showing grossly so striking an anomaly. As a result there have been few contributions to the histology of the malformation. Trillat and Pigeaud [18] reported that their studies showed both portions of the esophagus to be histologically normal. However, most others who made microscopic examinations reported varying types of histologic changes. Ballantyne [5] stated that normal

17. Reynolds, R. P., and Morrison, W. W.: Congenital Malformations of the Esophagus, Am. J. Dis. Child. **21**:339, 1921.

18. Trillat and Pigeaud: Un cas de communication entre l'oesophage et la trachée-rohres, Bull. Soc. d'obst. et de gynèc. **17**:75, 1928.

stratified squamous epithelium was found in both the upper and the lower portions of the esophagus, and cited Luschka as having found striped muscle in the upper portion and only unstriped muscle in the lower. Fourteen specimens were collected and examined by Keith.[14] He believed that the break in the esophagus occurred at what he considered was the junction of two parts of different origin. He considered that the upper part was pharyngeal. It was almost entirely composed of striated muscle fibers. He considered the lower part as having been derived from the primitive esophagus. This was composed of nonstriated muscle fibers. These histologic observations are, of course, a confirmation of Ballantyne's [5] statement. Latz [19] reported a case in which the muscle bundles of the upper part of the esophagus not only were composed exclusively of the striated type, but were all longitudinal in their course. The musculature of the lower portion was composed of smooth muscle.

A few histologic studies have shown abnormalities in the structure of the trachea, predominantly in the region of the fistula. Ladwig [20] reported a case in which the lower portion of the esophagus was seen to continue in the posterior wall of the trachea. Thick circular and longitudinal layers of muscle and glands, similar to those found in the esophagus, were found in the trachea. In this case he could demonstrate stratified squamous epithelium continuing in the posterior wall of the trachea, and its extension to the thyroid cartilage. He therefore considered the posterior wall of the trachea to be in the nature of an esophageal wall. Konopacki [16] also found squamous epithelial cells in the mucosa of the trachea in the region of the fistula. He also found an abnormal thickness of the musculature of the trachea, which contained several cross-striated fibers. Gutmann [21] stated that he found fibers of the posterior wall of the esophagus continuing for a distance in the anterior wall of the trachea.

Summary of the Microscopic Structure of Four Specimens Reported Here.—The upper culdesac was of a uniform structure in the four cases examined. The epithelium was of the normal stratified squamous variety. The muscularis mucosa was irregularly slightly hypertrophied. Occasionally, it was seen to be present as a very thin layer of smooth muscle, and it was actually absent in a region in one of the sections. The submucosa was present as a definite layer in the structure of the wall and contained few mucous glands. The musculature showed the most marked

19. Cited in Henke and Lubarsch (footnote 4).

20. Ladwig, A.: Ein bermerkenswerter Fall von Missbildung des Oesophagus-Tracheal-Rohres, Centralbl. f. allg. Path. u. path. Anat. **31**:613, 1920.

21. Gutmann, S.: Ueber einen Fall von Oesophagusatresie und Oesophago-Trachealfistel, Frankfurt. Ztschr. f. Path. **9**:459, 1912.

changes. It was regularly considerably hypertrophied and appeared normal in thickness only when the upper sac was considerably dilated. Although it was in general composed of an outer longitudinal and an inner circular layer, this arrangement was frequently distorted, and in one section it was reversed for a short distance. The hypertrophy of the musculature was accompanied by overdevelopment of Auerbach's plexus. The musculature was composed mostly of striated skeletal muscle, especially the outer longitudinal layer. As one would expect, the proportion of smooth muscle increased in amount as one proceeded caudadly. This was true especially of the inner layer. The outer longitudinal layer, however, generally consisted of the striated type to the very tip, with the exception of the anterior portion, which consisted mostly of smooth muscle. The anterior wall was seen to fuse with the smooth muscle of the posterior membranous wall of the trachea. A large amount of fibrous tissue was present within the musculature, being so marked in areas as to interrupt completely the course of the muscle fibers.

With the exception of its fistulous end, the lower portion of the esophagus was normal in structure. At this site, the esophagus had essentially a tracheal structure. The mucosa in three of the specimens was of the tracheal type and in one was of an undifferentiated type. In every instance, mixed glands were seen to be dispersed among the muscle bundles of the esophagus in this region. Study of sections demonstrated that the normal musculature of the lower portion of the esophagus became irregular as it approached the trachea. The arrangement of normal esophageal musculature, which is composed of inner circular and outer longitudinal layers, became irregular as it approached the region of the fistula and continued as a longitudinal layer continuous with the smooth muscle of the posterior membranous wall of the trachea. Other than the fistulous opening, no abnormalities were noted in the structure of the trachea.

Because of the presence of tracheal structures in the upper end of the lower portion of the esophagus and the change in musculature of the upper sac from the striated to the nonstriated type in its anterior wall proximal to the trachea it was thought that both sections of the esophagus shared in the tracheal structure. The musculature of the upper sac was interpreted as being similar to that of the membranous wall of the trachea to which it was fused. However, examination of apparently normal esophagi and tracheae in four infants of approximately the same age presented this alternation of smooth and striated muscle to almost the same degree. Nevertheless, as shown in the previous paragraph, the fistulous end of the lower segment of the esophagus had a tracheal structure. This observation was contrary to those reported previously, in which esophageal elements were found in the trachea and no tracheal

elements in the esophagus. If one reviews the histologic descriptions in all the reports, one notices that there has been a lack of uniformity in the microscopic structure of the fistulous site. Even the trachea has been reported to show abnormalities in its histologic structure.

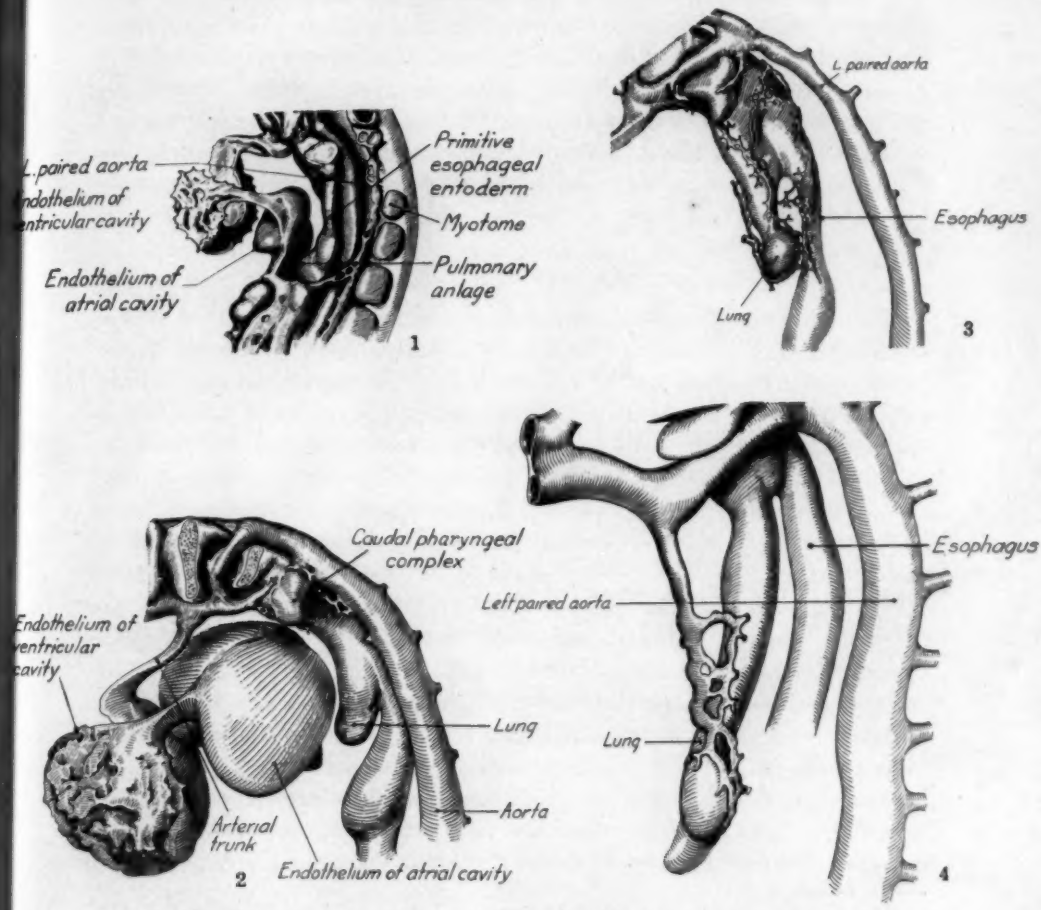

Fig. 2.—Embryos of 3, 4, 6 and 11 mm., illustrating the stages in the normal development of the esophagus and trachea (modified from Congdon, E. D.); Contrib. Embryol., Carnegie Inst., Washington **14**:47, 1922.

NORMAL DEVELOPMENT OF ESOPHAGUS AND TRACHEA FROM THE PRIMITIVE FOREGUT

A brief résumé of the normal development of the esophagus and trachea from the primitive foregut may aid in the interpretation of the various theories advanced. In the 3 mm. embryo (fig. 2) the first anlage of the respiratory system (pulmonary anlage) appears caudal to the pharyngeal pouches as a ventral rounded mass of entoderm. In

the 4 mm. embryo growth of the anlage has resulted in caudal prolongation and in the formation of two lateral longitudinal grooves, of which the corresponding internal elevation is the anlage of a septum. In this stage the trachea is seen to be already partially separated from the esophagus. Reference to the diagrammatic figure will illustrate the appearance of a cross-section of this region at this stage. The separation is more definite in the 5 mm. embryo and practically complete in the 11 mm. embryo. To many authors, the apparent cephalad growth of the ventral transverse groove between the lung anlage and the esophagus, along with the deepening of the longitudinal lateral grooves, constitutes a tracheo-esophageal septum which ultimately effects a complete separation in a cephalad direction.

GENESIS OF THE ANOMALY

Many theories have been advanced to explain the congenital anomaly considered here. Luschka [22] and independently Klebs [23] held that fetal inflammation or intra-uterine trauma played the important rôle. The marked constancy of the malformation makes any theory that has to do with inflammatory or traumatic factors seem unlikely. In the theories proposed by Robin,[24] Leven [25] and Regnier,[26] the upper portion of the esophagus or the entire esophagus is considered as ectodermal. This it most certainly is not. A study of early embryos demonstrates the epithelium of the entire esophagus to be derived from entoderm. Klebs,[23] and later Kraus,[27] advanced the conception that too much of the original formative material was used up in the development of the trachea, bronchi and lungs, so that an atresia of the esophagus resulted. Shattock's [28] explanation is somewhat similar. He believed that the posterior wall occasionally participated in the formation of the respiratory apparatus and was brought forward, thus narrowing the lumen. This forms the basis for his explanation of the development of the anomaly. The remaining theories are those that are most widely accepted, and may be grouped under three headings.

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22. Luschka, cited by Zeit, F. R.: Congenital Atresia of the Esophagus, J. M. Research **22**:45, 1912-1913.

23. Klebs, cited by Zausch, P.: Ein Fall von Oesophagusatresie und Oesophago-Trachealfistel, Virchows Arch. f. path. Anat. **234**:94, 1921.

24. Robin, cited by Rogier (footnote 13).

25. Leven, cited by Gutmann (footnote 21).

26. Regnier, cited by Trillat and Pigeaud (footnote 18).

27. Kraus, cited by Ysander, F.: Zur Frage der Genese der Oesophagusatresien, Upsala läkaref. förh. **30**:195, 1924.

28. Shattock, S. G.: Congenital Atresia of the Esophagus, Tr. Path. Soc. London **41**:87, 1890.

Theories Dealing with Retardation of Esophageal Development at a Time When the Esophagus Is Still Solid.—Kreuter [29] stated that to the fifth week the esophagus is a tube. From this period on, intensive epithelial proliferation results in the lumen being reduced to a slit. The most marked proliferation appears at the region of the bifurcation. Later vacuolization of the epithelium causes a dissolution of the atresia, so that finally a permanent lumen is formed. Permanence of the early embryonal phase in which the esophagus is solid causes the atresia. The epithelium perishes and is replaced by connective tissue. This theory was apparently corroborated by Reese,[30] who stated that temporary closure of a part of the anterior region of the digestive tract is commonly observed in the various classes of lower vertebrates in early embryonal life. The work of Schridde [31] on fifty human embryos constitutes a direct contradiction to Kreuter.[29] Schridde found the esophagus to have a lumen at all periods of its development. There are no vacuoles; what appear to be vacuoles are epithelial bridges due to epithelial proliferation in circumscribed regions. In the region where the atresia is formed, the epithelial bridges are not present. He believed that Kreuter's conception was a misinterpretation of the facts. Further, as pointed out by Forssner,[32] a study of embryos demonstrates that the separation of the trachea from the esophagus occurs when the embryo is at the early stage of about from 4 to 5 mm., whereas the so-called epithelial obliteration occurs at the much later stage of about 20 mm. It is unlikely that the anomaly develops at more than one stage in the formation of the trachea and esophagus.

Theories Concerning Deviation of the Tracheo-Esophageal Septum.—According to Keith and Spicer,[33] the lateral tracheo-esophageal ridges and folds normally proceed horizontally backward so as to meet between the pulmonary buds and the esophagus and so divide the primitive esophagus into a dorsal and a ventral portion. To produce this anomaly they must proceed obliquely backward and dorsalward so as to meet on the dorsal wall of the foregut. Ribbert [16] also believed that the lower part of the separating septum becomes attached to the posterior wall of the foregut, thus causing the atresia. Konopacki and Hoffman [34] advanced

29. Kreuter, cited by Schridde, H.: Ueber Epithelproliferationen in der menschlichen Speiseröhre, Virchows Arch. f. path. Anat. **191**:178, 1908.

30. Reese, A. M.: The Occlusion of the Oesophagus and Trachea in Crocdillia and Snakes, Am. J. Anat. **37**:195, 1926.

31. Schridde, H.: Ueber Epithelproliferationen in der menschlichen Speiseröhre, Virchows Arch. f. path. Anat. **191**:178, 1908.

32. Forssner, cited by Schmitz, J. A.: Ueber de formate Genese der Oesophagus Missbildungen, Virchows Arch. f. path. Anat. **247**:278, 1923.

33. Keith, A., and Spicer, J. E.: Three Cases of Malformation of the Tracheo-Esophageal Septum, J. Anat. & Physiol. **41**:52, 1906-1907.

34. Konopacki and Hoffman, cited in Abt, Isaac A.: Pediatrics, Philadelphia, W. B. Saunders Company, 1924, vol. 3, p. 407.

a similar theory. They assumed the lateral crests which should separate the respiratory apparatus from the esophagus to deviate from the normal pathway in an oblique course. To Zeit,[35] arrested development of the lateral ridges, which unite incompletely in the midline, explains the fistula and a "faulty anlage" the atresia. Lewis [36] believed that the lower portion of the tracheo-esophageal septum fails to develop, thus leaving the esophagus in communication with the lower part of the trachea. He called attention to a lateral furrow of the wall situated externally on either side of the esophagus caudad to the ventral pulmonary anlage and presenting a corresponding internal elevation. This same depression was seen in reconstructed models of the entoderm of 4 mm. embryos in the collection of the Carnegie Institution of Washington. It is so situated that if the walls of the esophagus should coalesce along this groove a ventral portion would be cut off, communicating freely with the trachea near its bifurcation. The part of the esophagus dorsal to this groove has a narrower lumen than the ventral part, and to produce the anomaly this portion must become occluded. Giffhorn [9] advanced two explanations. One is similar to that of Keith and Spicer,[33] and the second is based on a pushing forward of the posterior wall to meet the lateral folds in a manner similar to that explained in Shattock's [28] theory. As stated, Ladwig [20] found esophageal elements in the posterior wall of the trachea and therefore considered the posterior wall of the trachea as the anterior wall of the esophagus, and also held that the fact that the separation of the trachea and the esophagus exists in the upper portion and not in the lower meant that separation proceeds caudad, not cephalad. His histologic observations will be interpreted in a different manner in a later discussion. That normal separation proceeds in a cephalad direction has been shown here, and there is no evidence that Ladwig's interpretation is true.

Theories Concerning Changes of Tension.—Theories concerning changes of tension are among those most recently advanced and appear almost entirely in the German literature. Zausch,[37] a pupil of Beneke, advanced his mechanical theory. To him, the direction of pressure exerted by the heart anlage determines the type of anomaly produced. Thus, if it pushes in a cephalad direction, it prevents proper closure of the palatal clefts and results in cleft palate. If it is directed more pharyngealward, the pressure would act on the "narrow isthmus" of the esophageal anlage which overlies the heart, resulting in esophageal atresia and tracheo-esophageal fistula. In a case that he observed, an

35. Zeit, F. R.: Congenital Atresia of the Esophagus, J. M. Research **22**:45, 1912-1913.

36. Lewis, cited by Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1910, vol. 2, p. 312.

37. Zausch, P.: Ein Fall von Oesophagusatresie und Oesophago-Trachealfistel, Virchows Arch. f. path. Anat. **234**:94, 1921.

associated bifid uvula was interpreted as a palatal cleft, and both were conceived to be caused by pressure of the heart anlage and marked cervical curvature. Schmitz [38] also considered the primary cause to be pressure from the heart anlage. He called attention to the fact that the primitive foregut consists of only one layer of cells dorsally and three layers ventrally, with undifferentiated mesenchyme around it. The posterior wall not only is subject to the most tension, but is also the least resistant. If abnormal pressure continues, the posterior wall ruptures. The upper end becomes dislocated cranially, and the caudad end is kept in its original position by the heart anlage acting like a wedge. The epithelial cells in the ruptured end regenerate, but they cannot produce union, and hence atresia results. The pressure of the heart on the lower end leads to fusion of its posterior wall with the longitudinal lateral crests and results in a fistula.

Ysander [39] described an 8 mm. embryo of a monster of the type thoracopagus tetrabrachius in which there was but one stomach and one heart anlage, while the esophagus and the respiratory apparatus were duplicated identically. Each presented the typical esophageal atresia with tracheo-esophageal fistula seen in postnatal cases. This is the youngest embryo reported showing the anomaly, the only other being an 18.1 mm. embryo in the Harvard collection, which is single and similar to the postnatal type. Ysander explained the esophageal anomaly in a manner somewhat similar to that of Zausch [37] and Schmitz.[38] The foregut is bent in a curve by the large heart complex. The strain is put mostly on what he terms the periphery. This evidently is the dorsal wall of the primitive tube at about the midregion. This causes a scarcity of formative mass in this region, which results in a relative shifting of the lateral crests dorsalward, causing an atresia above, and a disappearance of the esophageal tube at the point of strain. The esophageal portion not only has lost the possibility of forming a tube here, but remains in communication with the trachea. Anders [40] agreed to these explanations assigning the important rôle to pressure.

According to several authors, pressure of adjacent arteries plays an important rôle in the formation of the anomaly. Thus the three cases presented by Keith and Spicer [33] showed a deep origin of the right subclavian artery. This abnormal artery crossed to the right side between the two parts of the esophagus. The right dorsal aorta has

38. Schmitz, J. A.: Ueber die formale Genese der Oesophagusmissbildungen, Virchows Arch. f. path. Anat. **247**:278, 1923.

39. Ysander, F.: Zur Frage der Genese der Oesophagusatresien, Upsala läkaref. förh. **30**:195, 1924.

40. Anders, H. E.: Die Genese der angeborenen Stenosen und Atresien des menschlichen Darmkanals im Lichte der vergleichender Entwicklungsgeschichte, Ergebn. d. Anat. u. Entwcklngsgesch. **26**:343, 1925.

been said to play a part (Schmerz [41]), and according to Kraus (cited in Ysander [39]) the cases in which a muscular cord unites the two sections of the esophagus are due to fetal atrophy from pressure of arteries.

Critique.—In an attempt to explain the altered condition, several authors found it necessary to carry the origin of the anomaly back to an early stage, that of the embryo of from 4 to 5 mm. But even this is not early enough. Many have noted the marked constancy of the morphology of the anomaly. The eight specimens described in this paper closely resemble each other. This constancy is in itself a sign of an early fundamental change. Later changes in the embryo are more likely to result in spotty anomalies that do not conform to a definite pattern. Further, this change may even rest on a genetic basis. Mackenzie is cited as having encountered a father all of whose children, by three wives, were born showing the anomaly. This is striking and, of course, can be explained only on a genetic basis. Ysander's [39] interesting 8 mm. embryo in which the typical anomaly was found bilaterally points also to an early inherent change.

The importance attributed by Zausch,[37] Schmitz [38] and Ysander [39] to the pressure of a large cardiac anlage with or without marked cervical curvature of the primary foregut is suggestive. But this hypothesis does not conform to the anatomic facts revealed by a study of embryos of the stage in which the separation of the esophagus and treachea occurs. Firstly, a study of reconstructed models of human embryos from 3 to 5 mm. in length shows the cardiac anlage always large, but still more important is the fact that a definite distance exists between cardiac anlage and primary foregut. Secondly, only a slight curvature can be seen in that portion of the primary foregut from which the anlage for the respiratory apparatus arises ventrally, namely, the region of the later bifurcation. A more marked curvature is seen in the reconstructed entoderm in the region of the last branchial cleft, but this area is far above the one under consideration. Thirdly, as even Schmitz pointed out, it is difficult to see why the trachea should not also be more directly involved, since it is even closer to the cardiac anlage. And lastly, malformations of the heart, other than the common patent foramen ovale and ductus arteriosus, are rarely seen accompanying the anomaly, and it is difficult to conceive of a large cardiac anlage acting abnormally which yet ultimately develops into a normal heart.

It is even more unlikely that pressure from an abnormal blood vessel causes the malformation. According to Dr. Streeter's conception, as a general rule, the growth of the vascular system does not influence the development of proximal tissues, but is to the contrary influenced by them. As pointed out in Keibel and Mall's "Manual of Human Embryology," [36] in the 4 mm. embryo, which is close to the stage in

41. Schmerz, H.: Die Chirurgie, Berlin, Kirschner & Nordmann, 1928, vol. 4.

which the anomaly arises, the arteries are not near this portion of the esophagus. The dorsal aortas run on each side of the primitive entoderm in this stage, and cannot conceivably influence the development of the esophagus in a manner to produce this anomaly. It is even more unlikely that any component of the aortic arches plays a rôle, for they are ventral and considerably cranial to the site of the malformation. It is more probable that the reported association of an abnormal vascular system in this region is an effect of the anomaly and not the cause of it.

The production of an anomaly of this type rests on altered or deficient growth of cells. A consideration of abnormal planes of cleavage, purely on a mechanical basis, as is encountered in some of the theories in which emphasis is laid on a deviated tracheo-esophageal septum, disguises this concept. As has been stated, embryologic study reveals no evidence for those factors that are alleged to be responsible for a deviated or altered septum, namely, pressure of a large cardiac anlage, an exaggerated cervical curvature or an abnormal vasculature. Unless

Fig. 3.—Diagrammatic representation of the neural tube of the embryonic spinal cord (*A*) and of the embryonic tracheo-esophageal tube (*B*) before the proliferation of cells that results in the closure of the former and the formation of two tubes from the latter.

concomitant primary changes are brought into play, most proponents are at a loss to explain why this abnormality of the tracheo-esophageal septum occurs. Dr. George L. Streeter suggested a simple analogy and conception of the development of the anomaly. If one first considers the formation of the common anomaly spina bifida, the explanation of the formation of the tracheo-esophageal fistula will be clearer. In the stage in which the neural tube is open, closure takes place not by the mesenchyme pushing in the entoderm at *b* and *b'* (fig. 3*A*), but by cellular proliferation at *a* and *a'* which later results in fusion. If for any reason there exists some deficiency in the cells at *a* and *a'* resulting in altered proliferation and nonunion, the open neural tube persists, and spina bifida results. The formation of the tracheo-esophageal fistula may be similarly considered. The two tubes, namely, a dorsal esophagus and a ventral trachea, are formed normally from one tube as the result of cellular proliferation at opposite lateral internal surfaces, *a* and *a'* (fig. 3*B*), and not as a result of mesenchyme pushing

the opposing lateral surfaces together at *b* and *b'*. If now for any reason a deficiency in the cells at *a* and *a'* causes altered proliferation and nonunion, a fistula between the trachea and the esophagus remains. The deficiency in the dorsal entodermal cells that were to give rise to the esophagus directly above this site may have resulted in its nonformation. Thus atresia of the esophagus results. There is some evidence that the entoderm shared in this disturbance, not only in this region but also by that over a wider area and even the surrounding mesenchyme. The abnormal histology of the tracheal and esophageal mucosa and the increased irregular musculature described under histology may be considered in this light.

If one begins with the 4 mm. embryo and traces the entoderm that is later to give rise to that portion of the esophagus dorsal to the pulmonary anlage, which is most concerned in the production of this anomaly, back through reconstructed models of earlier embryos it will be seen that in a presomite embryo the anterior termination of the neurenteric canal is in direct proximity to this portion of the entoderm. This relationship may conceivably play some rôle. In one of Dr. Streeter's embryos the neurenteric canal can clearly be seen to take an oblique course through the notochord from a dorsal caudad region to a ventral cephalad area in the entoderm. But whether the neurenteric canal plays any rôle or not, the primary change, according to the explanation presented here, is an early fundamental one that results in altered or deficient cell growth. This interpretation of the morphogenesis is not so different from those that concern altered septums and clefts. It differs markedly, however, from those in which the anomaly is ascribed to a mechanical cause. The etiologic factor is a fundamental change that rests probably on a genetic basis and not in primary concomitant associated abnormalities, such as an anomalous cardiovascular system.

SUMMARY

The gross structure of 8, and the microscopic structure of 4, cases of congenital atresia of the esophagus are described, with a review of the literature.

Reports on the histologic structure of this anomaly are infrequent. Microscopic examination in the cases presented revealed tracheal structures in the fistulous end of the lower segment of the esophagus. Although the gross structure of the anomaly is markedly constant, the microscopic structure, according to the histologic reports, presents marked variations.

The development of the anomaly seems to rest on an early fundamental change in the entodermal cells that are to give rise to the esophagus, and not on primary concomitant abnormalities. This change may be genetic and related to the anterior end of the neurenteric canal.

RETICULOSIS *

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AND
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Among the lesions of the hematopoietic apparatus, the changes involving proliferation of the reticulum cells have recently aroused increasing interest. Tumors of bone, single or multiple, deriving from endothelial cells as a matrix are well known, and as to their classification we refer to Ewing's work. There occurs, however, a rarer form of bone lesion in which the cells involved are apparently of a different type, as there is not the slightest tendency toward formation of vascular structures. These cells are also morphologically different from those described as occurring in the endotheliomas of bone. These lesions occur occasionally in connection with changes in other parts of the hematopoietic system, for example, in the lymphatic tissue, the spleen and the liver. They are accompanied in some instances by an invasion of the blood stream by immature leukocytes.

The number of such cases, either of the leukemic or of the aleukemic type, in which systemic lesions of the hematopoietic system could be established is rather limited; moreover, this question is of such theoretical importance that a detailed description of the following case seems justified.

REPORT OF CASE 1

A man, 75 years old, admitted to the hospital on account of injuries to his right shoulder and hip from falling, died the next day. There was found an intratrochanteric fracture of the right femur. All the bones of the pelvis and of both shoulders, the skull and the spine showed considerable mottling on roentgen examination. The roots of the lungs were infiltrated.

Autopsy.—At autopsy, the spleen was considerably enlarged and firm. The capsule was thick and milky. On section, it was smooth and showed a peculiar pattern, due to irregular, pale, pinkish-yellow and often coalescing areas in the pulp. The trabeculae stood out well. The follicles were hardly visible.

The liver was of medium size and rather hard, the surface smooth and the capsule thin and transparent. On section, a fine trabecular pattern of white color was noted. It spread irregularly over most of the reddish-brown parenchyma.

The abdominal lymph nodes were enlarged, the largest occurring about the portal region. They were soft, and on section were finely granular and white. Other enlarged nodes were found in front of the vertebral column and in the right inguinal region.

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Wedges cut out from the lumbar vertebrae showed the substitution of the spongy bone by a compact yellowish tissue. In between there were scattered reddish areas, containing islands of spongy bone with marrow. The ribs also showed substitution of their spongy structure by compact, sclerotic, yellowish-white tissue.

The neck of the right femur was fractured. The surrounding soft tissues were infiltrated with blood. There was a cavity in the neck of the femur about the size of a goose egg extending upward to the head on one side and downward to the greater trochanter on the other. The adjacent bony tissue was brittle, and between the fragments of the broken wall of the cavity was soft, moist, dark red tissue. Below the cavity, the shaft showed substantial, compact bony cortex with an unusually wide marrow cavity, filled with a rather solid, grayish-red marrow.

Histologic Examination.—The tissue infiltrating the bones in the vicinity of the fracture, as well as in the ribs and vertebrae, consisted of spindle-shaped cells with ample cytoplasm and bipolar processes that occasionally seemed to continue into fibrils. The nuclei were elongated or oval and vesicular, and contained fine chromatin granules. Between the spindle-shaped cells there were others, round and containing round or bean-shaped nuclei. There were also numerous myelocytes, many with eosinophilic granules, and mononuclear agranular cells that looked like myeloblasts. Typical bone marrow giant cells and occasional lymphocytes also occurred. Some of the tumor cells had larger, even giant nuclei, but they were always poorer in chromatin than the myeloplaques. Sections stained with Mallory's aniline blue-orange mixture revealed a fine network of pale-bluish fibrils interconnecting the spindle-shaped cells. Silver impregnation showed that these were reticulum fibrils.

In the spleen, very small atrophic follicles and heavy trabeculae were seen. The pulp was rather cellular in places, but presented also extensive areas of fibrosis. Brownish-yellow pigment was present and particularly abundant in the periphery of the fibrotic areas. Under high power, these fibrotic areas showed small, spindle-shaped cells embedded between hyaline fibrils. The cellular part also contained large collections of spindle-shaped cells, numerous polymorphonuclear cells and myelocytes, many of which were eosinophilic. The brown pigment was partly extracellular and partly inside the reticulum cells. Some of the splenic sinuses were obliterated; others were patent and lined by small cuboidal or flat cells. Mallory's stain showed a dense network of fibrils, coarse and dark blue in the fibrosed areas, and fine and pale purplish in the cellular areas. Silver impregnation revealed a dense network of fine argentophil fibers throughout the pulp, with coarse fibrils in the fibrous areas. The small lymph follicles were the only places free from these fibrils.

The lymph nodes presented a plexiform arrangement. The plexuses were separated by loose connectve tissue, holding many small blood and lymph vessels. Occasionally, the plexuses showed remnants of lymphatic tissue, particularly those at the periphery of the node. The preponderant cells, however, were the same, mostly spindle-shaped cells, described in the bone marrow. They grew sometimes in the form of parallel fibrillar strands. Silver impregnation showed a rather dense network of argentophil fibrils, in the meshes of which were embedded pale-yellowish reticulum cells. Groups of lymphocytes, where present, stained darker and showed almost no intercellular fibrils. Mallory's stain brought out the same points.

The hepatic cells contained a good deal of hemosiderin, especially those in the periphery of the lobules. The periportal connective tissue was rather cellular. There were also cellular foci without any relation to the periportal fields. Some of the smaller foci were apparently developed in the spaces between two hepatic trabeculae, while the larger nodules seemed to replace the hepatic tissue, as shown

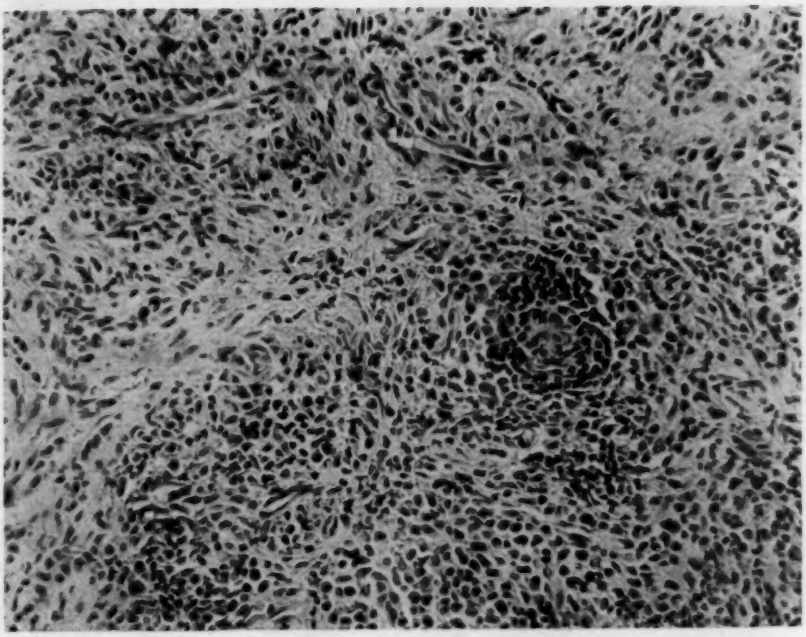

Fig. 1.—Spleen, showing remnant of malpighian follicle surrounded by spindle-shaped reticulum cells, and an adjacent area of fibrosis.

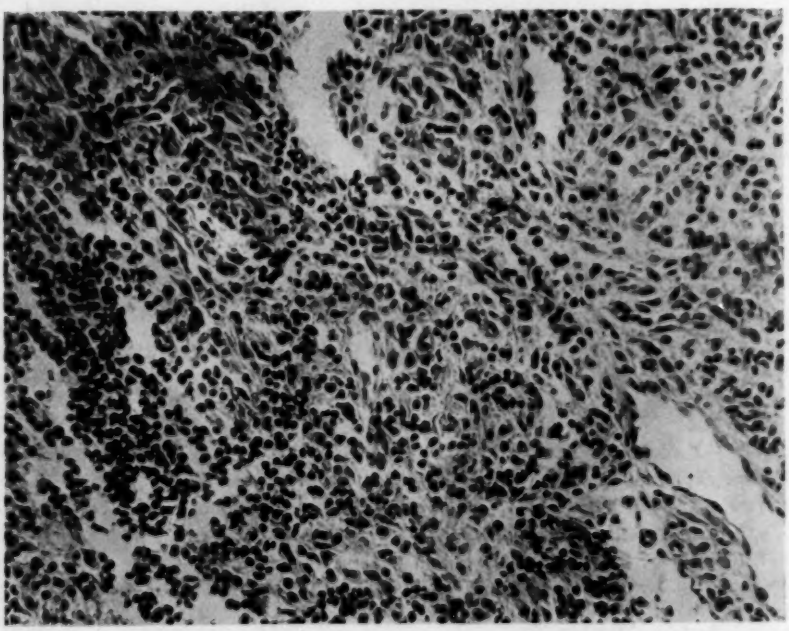

Fig. 2.—Lymph node, showing lymphatic tissue replaced by proliferated reticulum cells, and wide lymphatic sinuses lined with endothelium.

by occasional remnants of hepatic cells within the nodules. Most of the cells were of the spindle-shaped type, but in between there were also lymphocytes, polymorphonuclear cells, myelocytes and numerous eosinophils. Examination under the high power lens showed accumulations of elongated cells in the capillaries even at a distance from the larger nodules. These cells were either identical with, or resembled closely, Kupffer cells. In some capillaries, the Kupffer cells could be seen in the process of division, two or three of these cells taking the place of one. Other capillaries showed larger groups of cells, some of which were also larger in size. Still others were not only plugged by such cells, but also visibly distended by their accumulation until eventually the nodular stage was reached. Thus was seen an unbroken chain from the division of the single Kupffer cell to the formation of the grossly visible nodule.

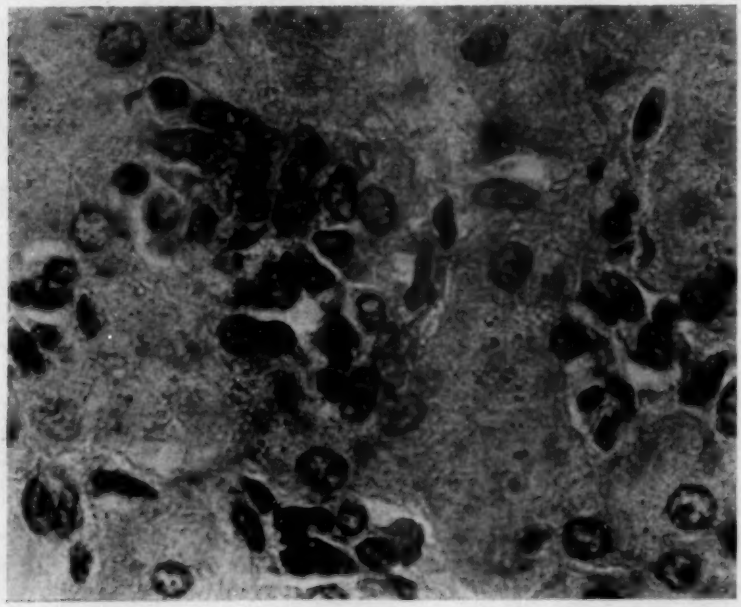

Fig. 3.—Liver, showing nodule formed by proliferation of Kupffer cells.

In searching the literature, we were able to find twenty-two similar cases.[1]

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1. Akiba, R.: Virchows Arch. f. path. Anat. **260**:262, 1926. Bock, H. E., and Wiede, K.; ibid. **276**:553, 1930. Borissowa, A.: ibid. **172**:108, 1903. Derischanoff, S. M.: Frankfurt. Ztschr. f. Path. **41**:184, 1931. Ewald, O.: Deutsches Arch. f. klin. Med. **142**:222, 1923. Feller, A., and Risak, E.: Folia haemat. **43**: 377, 1931. Goldschmidt, E., and Isaac, S.: Deutsches Arch. f. klin. Med. **138**:291, 1922. Goedel, A.: Frankfurt. Ztschr. f. Path. **29**:375, 1923. Hittmair, A.: Folia haemat. **37**:321, 1928. Letterer, E.: Frankfurt. Ztschr. f. Path. **30**:377, 1924. Paine, C. G.: J. Path. & Bact. **34**:139, 1931. Pentmann, I.: Frankfurt. Ztschr. f. Path. **18**:121, 1916. Reschad, H., and Schilling, V.: München. med. Wchnschr. **60**:1981, 1913. Richter, M. M.: Am. J. Path. **4**:285, 1928. Roulet, F.: Virchows

In addition to these systemic cases, we found a number of others in which only a specimen of tissue from a lymph node or a tonsil was examined.[2] The changes were comparable with those observed in the lymph nodes in some of the systemic cases. One case (Roulet's case 8) came to autopsy. It showed also involvement of the mediastinum, lungs and thyroid gland. Myelogenous leukemia seems to have been present in one other case (Roulet's case 9). We also observed a case of this type in which the clinical picture was suggestive of a systemic lesion.

REPORT OF CASE 2

A man, aged 35, complained of pain in the right hip, in both flanks, and in the epigastrium and of great loss of weight. The spleen was enlarged, and there was a chain of palpable glands in the neck. The temperature ranged between 101 and 104 F.; the urine contained casts and leukocytes; there were marked secondary anemia and leukocytosis with 13 per cent eosinophils and occasional myelocytes. Roentgen examination showed destruction of the body of the right os innominatum, internal to the acetabulum. The patient was discharged after one week and shortly afterward died.

Biopsy of a lymph node showed complete obliteration of the original structure. There were still lymphocytes left in the tissue, but they were irregularly scattered throughout. Instead there were irregularly distributed cells of odd shapes and varying sizes. Most of these were large, and their cytoplasm was more or less abundant; yet two different cell types could be distinguished: One assumed a pale stain and had fibrillar, not collagenous, stroma. The nuclei of these cells were vesicular and pale and occasionally showed nucleoli. The other cell type was conspicuous for the intense basophilia of the cytoplasm. It stained so intensely with hematoxylin that it was often impossible to distinguish the nucleus from the cell body. The nuclei were hypochromatic and oddly shaped. Some showed evidence of karyorrhexis; others showed atypical mitotic figures. Transition forms between the two major cell types were common.

The large basophilic cells had a tendency to form groups that were often almost trabecular. Some of them were also found within the lumen of the larger lymphatic spaces, which were lined with a flat endothelium or occasionally with cells that seemed to participate in the changes described.

Another very small lymph node showed almost normal structure with a few secondary follicles, except for an overabundance of lymph spaces which were lined with endothelium, often large and of peculiar appearance. There was also a definite hyperplasia of reticulum cells, many of which had taken up pigmented particles. A few scattered reticulum cells were conspicuous for their irregular hyperchromatic nuclei. Such cells were somewhat more numerous in the center of the secondary follicles.

Arch. f. path. Anat. **277**:15, 1930. Sachs, F., and Wohlwill, F.: ibid. **264**:640, 1927. Schultz, A.; Wermbter, F., and Puhl, H.: ibid. **252**:519, 1924. Swirtschewskaja, B.: ibid. **267**:426, 1928. Terplan, K.: Verhandl. d. deutsch. path. Gesellsch. **25**:69, 1930. Terplan, K., and Mittelbach, M.: Virchows Arch. f. path. Anat. **271**:259, 1929. Tschistowitsch, T., and Bykowa, O.: ibid. **267**:91, 1928. Ugriumow, B.: Centralbl. f. allg. Path. u. path. Anat. **45**:103, 1928.

2. Komocki, W.: Virchows Arch. f. path. Anat. **250**:517, 1924; **277**:605, 1930. Roulet, F.: ibid. **277**:15, 1930.

COMMENT

In reviewing the previously reported cases of similar character, we were impressed with one feature which was common in all of them as well as in our own: the proliferation of the reticulum cells in a manner that it is fair to call systemic. The most generalized condition was that described by Bock and Wiede, who observed a case of monocytic leukemia with infiltration of the liver, spleen and lymph nodes. The changes in the bone marrow were rather insignificant, which is the more remarkable as the proliferation of histiocytes throughout the connective tissue seemed to indicate a rather generalized activation of the mesenchyma.

The most frequently involved organ was the spleen, with the liver second. The bones and the bone marrow also showed changes in most of the cases. The lymph nodes, which were involved in the majority of the cases, were the main seat of the lesion in some of Roulet's series and in Goedel's case. It remains questionable whether the involvement was really systemic in our second case, in those of Komocki and in several others reported by Roulet. Only in some cases did the proliferation of the reticulum cells assume such proportions as to suggest a malignant neoplasm. These cases include our own second case, Schultz and associates', Richter's and three of Roulet's series.

It seems beyond reasonable doubt that the proliferating cells came from the so-called reticulum cells of the spleen, bone marrow and lymphatic tissue and from similar cells in the liver. The variety of appearance of these cells was noticed in most of the cases and brought out strikingly in ours. The cells were often spindle-shaped and showed a tendency to participate in the production of intercellular fibrils. The nature of these fibrils was not studied carefully in most of the cases. In our case, as shown by van Gieson's and Mallory's stains and by silver impregnation, the fibrils appeared to belong to the reticulum and not to the collagenous fibers. Goedel reported similar observations.

However, the spleen in our case showed areas of definite fibrosis, that is, the development of collagenous fibrils, which stained a bright red with van Gieson's stain and did not impregnate with silver. Borissowa described similar fibrotic areas in the spleen in her case. In view of the fact that gradual transition could be observed from the argentaffine reticulum to the areas of collagenous fibrosis, we are of the opinion that the fibrotic areas represented a late stage in the lesion. This would indicate that the pathologic reticulum cells may acquire fibroblastic properties in the sense that they produce collagenous connective tissue. A fibrotic replacement of structures previously built by reticulum cell proliferation is conceivable; yet our sections did not show any proliferation of fibroblasts, nor any such invasion of the

areas in question. The burden of the proof that reticulum cells do not form collagenous tissue falls on those who are not willing to admit this possibility.

The proliferated reticulum cells were also described by many authors as polygonal with scanty cytoplasm, yet showing processes that connected with the fibrillar network of the reticulum. Pentmann and others described the occurrence of giant cells in the lesions. We saw such cells in the bone marrow. Most of the authors believed that these giant cells were the result of incomplete amitotic division. They varied in size, were irregular, and contained as many as six nuclei with a fine chromatin network and distinct spherical nucleoli. The cell body showed indistinct outlines and coarse processes. The proliferation of these cells at times led to the formation of multinucleated protoplasmic masses of peculiar appearance.

Another type of cell that occurred in our case as well as in those of Akiba and Roulet was a large, round cell with round nucleus, which did not show fibrillar connection with the reticulum, and which was found free either in the meshes of the reticulum or in the open lumen of lymphatic and blood capillaries. This cell was, as a rule, distinctly basophilic. Its cytoplasm was in many cases more abundant than that of a myeloblast. Transitions between this round cell type and the spindle-shaped form were clearly demonstrable. It appeared to be a cell that had come from the same matrix, but that had lost its ability to produce reticulum fibrils. In other words, the cell was less differentiated and was reverting to the type of a more primitive mesenchymal cell.

The intertrabecular blood spaces in the liver showed extensive accumulations of cells, not only in the cases of leukemic type, but also in those in which the blood did not disclose evidence of leukemia. Moreover, proliferation of spindle-shaped cells was clearly demonstrable. Their morphology excluded the possibility that we were dealing with hematogenous cells. The question arises whether these cells were products of the lining endothelium of those sinuses, or whether they derived from other mesenchymal cells located in the intertrabecular spaces and not forming part of the endothelial lining. It hardly seems justifiable to distinguish between phagocytic mesenchymal (Kupffer) cells and lining endothelium. It is highly probable that the phagocytic stellate cell represents only a functional stage in the life cycle of the intertrabecular hepatic capillary endothelium.[3] We believe, therefore,

3. Goldzieher, M. A., and Peck, S. M.: Experimental Studies on the Reticulo-Endothelial System: I. Response to Infection, Arch. Path. **3**:629, 1927; II. Effect of Mercury Salts and Sulpharsphenamine on Reticulo-Endothelial Cells, ibid. **3**:635, 1927.

that the lining endothelium of the hepatic capillaries participates in the systemic proliferation of the reticulum cells.

Of special interest is the proliferation of the endothelium of the splenic sinuses, which was noticeable in several cases. The case of Pentmann is particularly noteworthy because the changes were accompanied by the formation of a large, cavernous, angiomatous nodule in the spleen and also in one of the vertebrae. These observations would indicate that the sinus endothelium in the spleen and liver is not as remote from the reticulum cells as it was claimed at one time to be. They represent, indeed, an instance of special differentiation that serves different purposes, and this is expressed by morphologic dissimilarity. Essentially, however, these cells are all derived from the same primitive mesenchymal cell. Although they represent products of diverging differentiation, still they belong to the same class of reticulo-endothelial cells and may participate in the changes which befall, as a rule, more conspicuously the reticulum cells proper.

The real nature of the lesion described is difficult to define. This is brought out by the diversity in the nomenclature. Some of the cases were described as granulomatous lesions and others as leukemia. Some authors chose the noncommittal term of hyperplasia of the reticulo-endothelial apparatus, while others selected the new terms of reticulosis, reticuloma and reticulo-endotheliosis. In some of the cases, the neoplastic character of the lesion was so striking that the term reticulosarcoma or retothelsarcoma was applied. This list of more or less suggestive names should not include the misleading terms used by some authors, such as angiosarcoma.

In our first case, the lesion of the bones was certainly suggestive of a malignant neoplasm, whereas the appearance of the spleen, lymph nodes and liver indicated a systemic involvement of hyperplastic rather than of neoplastic nature. It is well known how difficult it is to draw sharp lines of demarcation between cases of systemic involvement of the hematopoietic apparatus. We refer to the leukosarcoma of Sternberg, in which leukemia is associated with a growth of unquestionably sarcomatous behavior and microscopic appearance. We also refer to Hodgkin's disease, in which the systemic formation of granulation tissue is not infrequently associated with aggressive, sarcoma-like proliferation of the newly formed tissue. These cases were spoken of by Ewing as Hodgkin's sarcoma.

Roulet drew attention to the similarity between certain conditions described as lymphosarcoma and what he calls retothelsarcoma of the lymph nodes. It is obvious that many of the older authors, including Ghon and Roman,[4] described sarcoma of reticulum cells as lympho-

4. Ghon, A., and Roman, B.: Frankfurt. Ztschr. f. Path. **19**:1, 1916.

sarcoma. Most of these were not of a systemic type, while others approached a degree of dissemination that may bring them within the fold of systemic lesions. It is important to stress that none of the cases, with the exception of Pentmann's, showed any attempt at differentiation into structures of endothelial character. This applies also to Paine's case, although he mentioned a tubular structure, but one without definite lumen. His choice of name, "angiosarcoma," does not seem to be warranted by his own description. None of the lesions of the bone marrow cited in this paper showed any of the features characteristic of endothelioma of bone. The latter, and particularly Ewing's diffuse endothelioma, seem to come from cells that maintain a higher degree of differentiation, remaining specialized endothelial cells, notwithstanding their neoplastic tendencies and their derivation from the same matrix that is involved in the systemic process investigated in this paper. Careful comparison with several cases of Ewing's tumor has convinced us that it is impossible to differentiate histologically the tumor-like lesions in cases of systemic involvement of the reticulo-endothelium from Ewing's diffuse endothelioma of bone.

There are no definite criteria at present by which one can classify a lesion as neoplastic instead of hyperplastic. Thus it seems justified to stress the systemic nature of the lesion described and to designate it with the somewhat indefinite term of reticulosis, in order to distinguish it from related, but more localized and apparently more definitely aggressive lesions (reticulum sarcoma).

The relationship of the term reticulosis, in the sense applied by us, to the more commonly used terms, myelosis and lymphadenosis, may cause some misunderstanding. Both these terms are used instead of the ancient expression leukemia to designate a systemic disease in which myeloid or lymphatic cells are formed in excess, and in which many of these cells do not reach a stage of full differentiation and maturity. Both myelosis and lymphadenosis may or may not be accompanied by invasion of the blood stream, that is, by the clinical symptoms of leukemia. Essentially, however, both forms—whether leukemic or aleukemic—consist of a systemic hyperplasia of myeloid or lymphatic cells, respectively. Reticulosis, in contradistinction to both, represents hyperplasia of the third main constituent of the hematopoietic tissues.

It stands to reason that reticulosis may also occur in a leukemic form. The dominating cell type in these cases is the monocyte, and the literature on monocytic leukemia includes already a number of reports of well studied cases. The reticulo-endothelial origin of these cells was demonstrated in several of these cases by numerous transition forms in the circulating blood between mature monocytes and their stem cells, and particularly by the evidence that many of the proliferating reticulo-endothelial cells were being mobilized, since they were

found free in the lumen of hepatic or splenic sinuses. Richter described his case as one of lymphatic leukemia and Roulet his as one of myelogenous leukemia, yet without any details as to the blood picture or as to changes in the bone marrow. Thus it is impossible to decide whether these cases represent merely a coincidence of leukemia with reticulosis, or whether the leukemic blood changes were in some way based on proliferation of reticulum cells.

SUMMARY

A case of generalized proliferation of the reticulo-endothelial cells, with involvement of the bones, the spleen, the liver and lymph nodes, is described. Another case is described in which a comparable lesion developed from the reticulum cells of the lymph nodes. The relationship of both cases to others previously reported is discussed. The name reticulosis is suggested for this process, which may or may not associate itself with leukemia. Reticulosis cannot be always sharply demarcated from neoplastic conditions, and a number of cases remain on the borderline between reticulosis and reticulum cell sarcoma. Instances of the latter, which localizes in one place (lymph node or tonsil) have been previously classified as lymphosarcoma, Hodgkin's sarcoma, or endothelioma of lymph nodes.

THE GRADING OF CARCINOMA OF THE CERVIX UTERI AS CHECKED AT AUTOPSY*

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Although numerous attempts have been made to evaluate the clinical significance of histologic variations in malignant tumors,[1] the subject is still far from settled.[2] Various factors have been introduced in an effort to increase the accuracy of histologic grading. Some of these factors are anatomic, such as the location and the extent of the tumor, but clinical factors have been introduced as well, in an effort to maintain correlation between the grading and the end-result. Even economic status deserves consideration.

The inherent difficulties in any attempt to use duration of life as a criterion for the efficacy of the histologic grading of tumors are obvious. Thus, when any such scale is used, if it is to be at all accurate, it must allow for the normal duration of life in the untreated patient, the adequacy or inadequacy of treatment in the individual case, the fact that occasionally a patient dies as a result of treatment rather than of the disease itself and finally the fact that death may occur from entirely unrelated causes. Moreover, many of the patients do not die from carcinoma as such, but from related mechanical injuries, such as hydronephrosis.

A renewed incentive to attempts at grading was given by Ewing,[3] who pointed out that the histologic appearance often affords important clues to the radiosensitivity or radioresistance of a given growth.

Since most of the studies have used clinical criteria, particularly the duration of life after the onset of the disease, for the determining of the degree of malignancy, it has seemed worth while to attempt a study using largely pathologic data.

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The present study is based on the observations at autopsy in one hundred and two cases of carcinoma of the cervix uteri. The power of a tumor to metastasize has been taken arbitrarily as an index of its degree of malignancy. While perhaps the power of metastasis is a one-sided criterion of malignancy, its use has certain definite advantages. The personal equation is largely eliminated. Variations in type and efficiency of treatment are compensated for to some extent. Variations in clinical factors may be largely neglected. Intercurrent disease processes have little influence.

The primary tumors were graded; the sections were compared with the previous biopsy specimens, if any, and then the distribution of metastases was studied. A modification of Broder's classification was used, dividing the epidermoid carcinomas, on the basis of differentiation of the tumor cells, frequency of mitosis and relation of tumor to stroma, into three groups: group 1, of low malignancy; group 2, of medium

TABLE 1.—*Distribution of Cases by Types*

Type and Grade	Cases	Average Age of Patient at Death	Average Duration of Disease*
Epidermoid Carcinoma:			
Grade 1	23	53 yr., 7 mo.	2 yr., 9 mo.
Grade 2	44	51 yr.	2 yr., 1 mo.
Grade 3	18	45 yr., 4 mo.	1 yr., 2 mo.
Adeno-acanthoma	6	53 yr., 3 mo.	2 yr., 3 mo.
Adenocarcinoma	8	57 yr., 6 mo.	3 yr., 6 mo.
Cases in which all tumor was destroyed	3	47 yr., 7 mo.	1 yr.
Total	102	51 yr., 5 mo.	2 yr., 2 mo.

* Duration unknown, 2 cases.

and group 3, of high. In addition, the adeno-acanthomas (combined epidermoid and adenocarcinoma) and the adenocarcinomas of the cervix have been included.

In three cases, all of the tumor had been destroyed by the treatment and no metastases were found, death resulting in one of the cases from cachexia and in the other two from sepsis. These cases are particularly worthy of note, in that the short duration of life after treatment, averaging 7.6 months, would be considered as evidence of the presence of a highly malignant type of tumor.

In table 1, the distribution of cases by grade and type is shown, with the average age of the patients at death. As can be seen in table 1, a considerable proportion of the cases fall into grade 2, those of moderate malignancy. This is not because grade 2 is merely a scrap-bag for cases that do not fall definitely into group 1 or group 3, as only the cases are included in each group that meet the criteria for that group.

The average duration of all cases of the disease, both those in which there was treatment and those in which there was not, was 2.2 years.

In table 2 the types of treatment are given. It will be seen that the duration from the apparent onset of the disease to treatment is long in all cases, except the highly malignant cases of grade 3, the progress of which forced the patients to seek treatment within 7 months, on the average. In striking contrast are the three cases in which all the tumor was destroyed by treatment; in these the duration of symptoms before treatment had been only 4.7 months.

The biopsy specimens of the tumors that were entirely destroyed by treatment showed them to have been epidermoid carcinomas, grade 2.

TABLE 2.—*Types of Treatment*

		Epidermoid Carcinoma					Cases in Which All Tumor Was Destroyed
	Cases	Grade 1	Grade 2	Grade 3	Adeno-acanthoma	Adeno-carcinoma	
Total number of cases	102	23	44	18	6	8	3
Untreated	19	6	10	2	1	..	..
Treated by operation only	6	..	4	1	..	1	..
Treated by radium only	46	12	16	11	2	4	1
Treated by x-ray only	1	..	1	..	..	..	..
Treated by operation and radium	14	1	8	2	1	1	1
Treated by operation and x-ray	2	..	..	..	1	1	..
Treated by operation, radium and x-ray	3	1	1	1	..	..	..
Treated by radium and x-ray	11	3	4	1	1	1	1
Average duration until treatment*	11 mo.	1 yr., 1 mo.	11 mo.	7 mo.	11 mo.	1 yr., 9 mo.	5 mo.

* Duration unknown, 6 cases.

TABLE 3.—*Distribution of Metastases*

Type and Grade	Cases	Metastases Absent	Metastases to Regional Lymph Nodes	Metastases to Distant Lymph Nodes	Visceral Metastases	
					Cases	Percentage
Epidermoid Carcinoma:						
Grade 1	23	13	7	3	1	4
Grade 2	44	10	33	20	16	34
Grade 3	18	2	14	14	12	67
Adeno-acanthoma	6	..	5	3	4	67
Adenocarcinoma	8	3	5	3	2	25

This is in accord with clinical studies, which have shown grade 2 carcinomas of the cervix to respond best to irradiation.

Of minor interest is the frequent occurrence of vesicovaginal and rectovaginal fistulas in these cases. While of the forty-five patients in whom fistulas developed, thirty-two were radium-treated, there is no significant difference in the incidence of fistulas in the radium-treated patients and in the untreated patients or those treated with x-rays or operation, fistulas developing in 43 per cent of the radium-treated ones and 46 per cent of the others. In a few radium-treated patients, however, the character of the tissue surrounding the fistulas strongly suggested radiation necrosis as the cause for their presence.

The distribution of metastases is presented in table 3.

Of the epidermoid carcinomas, grade 1, thirteen, or 57 per cent, had no metastases, and only one, or 4 per cent, had visceral metastases. Of those of grade 2, only 23 per cent were without metastases; 77 per cent showed metastases to lymph nodes, and 34 per cent showed visceral metastases. Metastases were the rule with those of grade 3, only 11 per cent remaining localized, whereas 67 per cent had visceral metastases. Not one of the adeno-acanthomas remained localized, and again 67 per cent showed visceral metastases. The adenocarcinomas of the cervix are definitely less malignant than the adeno-acanthomas, 38 per cent remaining localized and only 25 per cent showing visceral metastases.

From these figures it may be seen that there is a close correlation between the histologic degree of malignancy of a tumor of the cervix uteri and its power to set up metastases. One epidermoid carcinoma of grade 1 and two of grade 2 developed in the retained cervix.

Six instances of multiple malignancy were encountered in the series: One was a very early adenocarcinoma of the fundus uteri, co-existing with an epidermoid carcinoma, grade 1, of the cervix; another was a carcinoma of the stomach, co-existing with an epidermoid carcinoma, grade 1, of the cervix; three were malignant adenomas of the rectum, two co-existing with an epidermoid carcinoma, grade 1, and one with an epidermoid carcinoma, grade 2, and there was one case of renal adenocarcinoma of the kidney co-existing with an epidermoid carcinoma, grade 3.

By far the commonest cause of death in the group was uremia resulting from ureteral obstruction, renal infection or a combination of the two. This caused one third of the deaths. Other important causes were cachexia, peritonitis, pneumonia, hemorrhage and intestinal obstruction.

SUMMARY AND CONCLUSIONS

A comparison of the distribution of metastases in one hundred and two cases of carcinoma of the cervix uteri in which autopsies were performed shows close correlation between the degree of malignancy as estimated by histologic grading and the distribution of metastases.

Metastasis is uncommon in epidermoid carcinoma, grade 1, and visceral involvement is very rare. In epidermoid carcinoma, grade 3, and in the adeno-acanthomas, metastasis to lymph nodes is the rule, and visceral metastases are common. So far as distribution of metastases is concerned, the degree of malignancy of epidermoid carcinoma, grade 2, and of adenocarcinoma of the cervix is approximately the same.

Impairment of renal function is the most common cause of death in cases of carcinoma of the cervix uteri.

EXPERIMENTAL PATHOLOGY OF THE LIVER

STUDIES III, IV AND V *

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III. THE EFFECT OF DIVERTING THE PORTAL BLOOD ON THE RESTORATION OF THE LIVER AFTER PARTIAL REMOVAL

One of the most outstanding characteristics of the liver is its restoration following injury or partial removal. Fishback [1] reviewed the previous work concerning this characteristic and presented our data with regard to it, which were collected from the observation of dogs, and Higgins and Anderson [2] reported the results of experiments on rats. The capacity of the liver to be restored after partial removal has been the main difficulty in producing a decrease of hepatic tissue and a decrease of function. It seemed of both clinical and experimental value to attempt to determine the cause or causes for such reaction, and whether any experimental conditions would impair the restorative ability of the liver after injury or loss of a portion. If this could be done, a method might be established for permanently reducing hepatic tissue and for studying reduced hepatic function. It might be of aid clinically in determining some of the factors on which such restoration occurs, and furnish a basis for the causes of the varied and characteristic changes found in cirrhosis. Several years ago, Mann and Magath [3] noted that restoration of the liver did not occur in an animal with Eck fistula. We shall present here more complete data concerning partial removal of the liver following diversion of the portal blood from that organ.

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* Submitted for publication, July 21, 1931.

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2. Higgins, G. M., and Anderson, R. M.: Experimental Pathology of the Liver: I. Restoration of the Liver of the White Rat Following Partial Surgical Removal, Arch. Path. **12**:186, 1931.

3. Mann, F. C., and Magath, T. B.: The Production of Chronic Liver Insufficiency, Am. J. Physiol. **59**:485, 1922.

Methods.—The experiments were carried out on dogs. All operative procedures were done under ether anesthesia, and with the employment of sterile technic. The portal vein was anastomosed to the vena cava by the modified cutting suture of Fischler and Schröder [4] as developed in our laboratory and as described by Fishback. After the Eck fistula had been made, the animals were carefully observed. These animals do not maintain nutrition well unless special care is exercised in their diet; it has been found that they do best on a diet of milk and syrup without meat or other concentrated protein. From 2 to 3 months after the portal blood had been diverted, two lobes of liver were removed. The technic of this procedure has been described by Fishback. A minimum of 30 per cent of the organ was removed. It was found that the intravenous administration of dextrose for several days following operation was a distinct benefit. The same care with regard to diet was necessary after the liver had been partially removed as after the Eck fistula had been made. Even with the utmost care some animals did not do well, and frequently 3 or 4 weeks after the second operation a duodenal ulcer developed and perforated. In some experiments, exploration was carried out at frequent intervals; the liver was examined for evidence of restoration and a specimen was removed for histologic examination. In other experiments, the animals were killed at stated intervals after the second operation, and the condition of the liver was noted.

Results.—Our observations of the effect of Eck fistula on the liver are similar to those described by Whipple and Hooper.[5] From six to ten weeks after diversion of the flow of portal blood, the liver assumed a characteristic condition. On gross examination, the most striking changes were in size and color. The liver of an animal with Eck fistula atrophied to about half its normal size. Its shape and contour remained unchanged, but there was a decrease in all dimensions to about half the normal, and it became characteristically pale, mottled and yellow. Microscopic examination revealed atrophy of the central portions of all lobules, with marked accumulation of fat in the hepatic cells of these regions. Frequently the accumulation of fat was so great that only a rim of the protoplasm of the hepatic cell with a nucleus pushed to the periphery of the cell remained. Although changes occurred first in the central portion of the lobule, they were not always confined to this region, but extended also by ramification to the periportal region. After the atrophy had reached a certain point, it appeared to remain stationary.

After the liver had been partially removed from the animal with Eck fistula, the remaining portion usually remained unchanged. This is in marked contrast with the remaining portion of the liver after partial removal from a normal dog, in which the edges of the remaining lobes become rounded and the lobes dome-shaped, and the total mass of hepatic tissue becomes approximately the same as before operation.

4. Fischler, F., and Schröder, R., quoted by Fishback, F. C.: Anastomosis of Portal Vein with Inferior Vena Cava; a Thoroughly Tested and Satisfactory Method of Making an Eck Fistula, Ann. Surg. **86**:436, 1927.

5. Whipple, G. H., and Hooper, C. W.: Bile Pigment Metabolism: VI. Bile Pigment Output Influenced by the Eck Fistula, Am. J. Physiol. **42**:544, 1917.

When the portal blood was diverted, the edges of the remaining lobes remained sharp, and the organ as a whole maintained its atrophic condition. In rare instances in which increase in size was noted, blood vessels carrying adhesions were always present. Microscopically, the liver of the animal with Eck fistula after partial removal differed little from its appearance before partial removal. In some experiments it seemed that there were fewer cells that appeared normal and more cells that contained fat.

Summary.—The portal blood was diverted from the canine liver by an Eck fistula, and after the liver had assumed the characterisics of Eck fistula, the effect of removing various amounts of the organ on the remaining portion was observed. Whereas partial removal of the normal liver is always followed by restoration of hepatic tissue, such regeneration did not occur when the portal blood was diverted.

IV. THE EFFECT OF PREVIOUS LIGATION OF THE COMMON BILE DUCT ON RESTORATION OF THE LIVER FOLLOWING ITS PARTIAL REMOVAL IN DOGS

There are many methods of injuring the liver; probably the most common clinical method is obstruction to the outflow of bile. The extent to which obstruction of the common bile duct affects the fundamental activities of the liver either transitorily or permanently has not been clearly indicated. The purpose in this research was to determine the effect of obstruction of the outflow of bile on one of the most outstanding reactions of normal hepatic tissue, that is, restoration after partial removal.

Methods.—All operations were performed on dogs under ether anesthesia, with rigid aseptic technic. The common bile duct was exposed, doubly clamped, cut and doubly ligated with linen about 1 cm. from its entrance into the duodenum; the duodenal end was not ligated. The gallbladder was removed, because it had been found that perforations of the cystic and common bile ducts are more common when the gallbladder becomes distended after obstruction to the outflow of bile. In order to have a specimen of normal liver for comparison, a triangular wedge of tissue was removed from the border of the right ventral lobe and placed immediately in Zenker's solution; the wound was closed with mattress sutures of catgut. The weight of the liver was estimated at this time from its size, from the size and weight of the dog, and from the table of weights of the livers of normal dogs, as outlined by Fishback. After operation the animal was placed on a diet of equal parts of milk and syrup, which had been found to be particularly valuable for the maintenance of animals with obstruction of the common bile duct. At a second operation, from 7 to 52 days after the obstruction of the outflow of bile, the liver was partially removed according to the technic described by Fishback. The resected lobes were weighed, and a specimen was fixed. The weight of the remaining portion was estimated. A minimum of 30 per cent of the organ was always removed, but usually the amount was greater. The appearance of the liver, the degree of dilatation of the common and hepatic bile ducts and the degree

of jaundice of the sclera and viscera were noted. The animal was again placed on a diet of milk and syrup until the termination of the experiment. Animals that did not die when under observation were killed by ether. Necropsy was always performed promptly. The abdominal and thoracic viscera were weighed separately to determine any increase in size. Ligation of the duct was verified, and the duodenum was opened to exclude the possibility of reestablishing drainage of bile. Tissue from the liver was fixed immediately in Zenker's solution. Sections were prepared and stained by the usual technic.

Results.—The length of life following ligation of the common bile duct varied greatly. Spontaneous death was usually due to rupture of a duodenal ulcer or to distention of the common bile duct, with peritonitis resulting. Jaundice, perceptible in the sclera from fourteen to sixteen hours after operation, increased rapidly, and reached its maximum within the first ten days. During this period the sclera, skin and mucous membranes became markedly yellow, and the viscera and other tissues, including the cartilage, yellowish to yellowish green. The intensity of the jaundice usually decreased in a few weeks, but normal color of the tissues never returned completely. In a few experiments a large amount of clear fluid was found in the abdomen from five to ten days after ligation of the common bile duct, but in general ascites was usual, although not marked.

At the second operation, the appearance of the liver was typical of that associated with obstruction to the flow of bile. It varied in color from greenish to brownish black and appeared distended and engorged; the individual lobules were prominent. The external surface was smooth and glistening, and frequently some adhesions were present between the duodenum and the under surface of the right central lobe. The common and hepatic bile ducts were usually dilated to about from four to six times their normal caliber, and their walls were thin and friable. The bile in the ducts was usually thin, watery and greenish brown. The microscopic picture consisted essentially of a slight increase in connective tissue in the interlobular spaces directly around the dilated bile ducts; this increase did not extend around the radicals of the portal vein or of the hepatic artery or into the lobule itself. The intrahepatic ducts were usually dilated to about twice the usual size, but the cells lining them appeared normal. In the interlobular spaces and throughout the parenchyma were numerous large and small areas of new bile ducts, which seemed to form whorls of new tissue throughout the organ; around these, connective tissue was slightly increased. The cells of the parenchyma disclosed little, if any, atrophy or other change, but those bordering the areas of new bile ducts were usually more deeply stained. Pigment was scattered irregularly throughout the organ, but apparently in the canaliculi rather than in the cells. Except for the presence of the pigment, the slight increase in connective tissue around the ducts and the areas of

new bile ducts, the organ appeared to be practically normal. There was no evidence of the formation of new cells before partial hepatectomy was performed.

The liver was partially removed from 5 to 52 days after ligation of the common bile duct; the average interval between the two operations was about 30 days. The immediate clinical effect after partial removal was slight. Several of the animals gained weight, were only slightly jaundiced and seemed to be in excellent health at the end of from 131 to 141 days after ligation of the common bile duct, or at the end of from 101 to 111 days after partial hepatectomy. Ascites was not marked when the prescribed regimen was maintained.

Several months after partial resection of the liver there appeared to be less engorgement than after ligation of the duct alone, and the color was possibly brownish red rather than greenish black; however, the organ did not differ strikingly from that in the earlier stages after simple ligation. The obstructed, uninfected organ had not increased in size or weight from 3 to 110 days after partial hepatectomy; this is in strong contrast with the almost complete regeneration of the unobstructed liver that has been demonstrated so often. In the fifth month, or more than 100 days after partial hepatectomy, the caliber of the ducts was about half what it was in the second month, and the walls were thick rather than thin and transparent, as in the earlier stages of distention. The character of the bile in the ducts was similar to that found earlier; namely, thin, watery and greenish brown, although occasionally white. Microscopically, in most cases, from 4 to 30 days after partial hepatectomy, the sinusoids were dilated and the cells of the parenchyma appeared somewhat swollen, but essentially normal. The picture was otherwise that of the liver at about the same interval following ligation of the common bile duct. In other experiments there was moderate proliferation of the cells of the parenchyma, with mitotic figures in various stages, but few in number. In the periphery of the lobule and especially around the whorls of new bile ducts, there were many areas composed of cells that appeared to be newly formed. The typical new cell was smaller than the normal parenchymal cell, and the protoplasm was stained a dark pink with hematoxylin and eosin. Its nucleus was elliptical or oval, very darkly stained and smaller than normal; a nucleolus was not seen. Later there was a more distinct increase in the connective tissue around the bile ducts, with a decrease in the pigment scattered throughout the organ.

Infection played an important part in the jaundiced dogs, and when it developed after either ligation of the common bile duct or partial hepatectomy, the animal did not live for more than a few weeks. When the infection occurred following ligation of the duct, the formation of connective tissue was much more marked than without it; when the infection was of a severe grade, atrophy of the parenchymal cells ensued

rapidly. Infection following partial hepatectomy produced the same changes in the remainder of the liver, but it is worthy of note that mitotic figures and new parenchymal cells were found in some livers in which abscesses were present, although the mitotic figures were not usually found in the lobes that contained the abscesses, and a few of the infected remaining lobes of liver showed a definite increase in size.

Comment.—The remarkable restoration of the normal liver after partial removal is in marked contrast with the effect of partial removal after obstruction of the common bile duct. Little, if any, increase in the size or weight of the liver was noted after the operation was performed at varying intervals following ligation of the common bile duct. Shortly after removal of a portion of a liver with obstructed biliary outflow, the hepatic sinusoids became engorged, and occasionally mitotic figures of the parenchymal cells were observed. These changes apparently signified some slight effort toward restoration, but the size or weight did not increase, or increased only to a slight extent; the edges of the liver remained sharp and did not become rounded as in the normal liver after partial removal. It is quite evident that restoration of the liver after partial removal does not occur or occurs only to a slight extent when outflow of bile is prevented.

Summary.—A series of experiments was performed on dogs to determine if the liver would be restored after partial removal if the outflow of bile had been previously obstructed. The routine procedures were as follows: (1) ligation of the common bile duct and removal of the gallbladder and (2) removal of two or more lobes of the liver at various intervals after the production of biliary obstruction. It was found that partial removal of the liver was not followed by the remarkable restoration that occurs in the normal liver.

V. THE EFFECT OF CIRRHOSIS ON RESTORATION OF THE LIVER AFTER PARTIAL REMOVAL

Previous observations of the restoration of the liver after partial removal have demonstrated that: (1) Removal of a portion of the normal liver is always followed by restoration; (2) if the portal blood has been diverted by an Eck fistula, restoration after partial removal does not occur, and (3) obstruction of the biliary outflow likewise prevents restoration of hepatic tissue after partial removal. Our purpose in this investigation was to determine if restoration of the cirrhotic liver occurs after partial removal.

Methods.—Dogs were used in all experiments. Cirrhosis was produced by the administration of carbon tetrachloride by stomach tube; this was done originally by Lamson and Wing.[6] The amount of the drug given varied, depending on its

6. Lamson, P. D., and Wing, Raymond: Early Cirrhosis of the Liver, Produced in Dogs by Carbon Tetrachloride, J. Pharmacol. & Exper. Therap. **29**: 191, 1926.

acute effect. The usual amount was from 3 to 5 cc. every 2 to 5 days. From 4 to 8 months and in most experiments 6 months after beginning the administration of the carbon tetrachloride, the animal was etherized, and with aseptic technic the liver was explored, the degree of cirrhosis was noted, and various lobes of the organ were removed. After permitting sufficient time for the restoration of the remaining portion of the liver as it occurs in the normal dog, either exploration was again carried out and the condition of the liver noted, or the dog was killed by bleeding under ether, the liver was examined and weighed, and sections were removed for microscopic examination.

Results.—The rate and degree with which cirrhosis developed following administration of the drug varied considerably in the different animals, but cirrhosis was found in all of them. When 30 per cent or more of the liver was removed with only a moderate degree of cirrhosis present, some restoration occurred in the remaining portion, as was evidenced by the rounded edges and dome shape of the lobes, as well as by the increase in weight of the entire organ. However, when cirrhosis was marked, the surface of the liver nodular, and the whole organ atrophic in association with a great increase in connective tissue, little, if any, restoration of the remaining portion occurred after partial removal. Except when cirrhosis was marked, however, slight restoration always occurred. There was more evidence of restoration in the cirrhotic liver than occurred following either diversion of the portal blood or obstruction of the outflow of bile.

Summary.—Cirrhosis of the liver was produced in dogs by the administration of carbon tetrachloride by stomach. When cirrhosis was well developed, the liver was partially removed. There was little restoration of the cirrhotic liver after its partial removal as compared with that of the normal liver. Restoration did not occur when the cirrhotic condition was well developed.

EFFECT OF HOMOLOGOUS MACERATED SKIN ON THE REGENERATION OF EPIDERMIS *

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Experiments were undertaken to determine the effect of macerated dead tissue on the regeneration of living cells of like kind maintained in contact with their normal blood supply. Many substances varying in character from complex tissue derivatives to simple inorganic salts have been applied to tissues proliferating in vitro. In the tissue cultures, in which the conditions of food supply are relatively simple, the mere addition or withholding of a substance from the medium may be sufficient for a determination of its effect on the cells. However, when a substance is placed within the animal, it may be diluted, absorbed, changed by the living cells with which it makes contact, or acted on by various enzymes. Also the cells on which the action of the substance introduced is to be tested are under the influence of factors that limit cell growth and control the regenerative processes of the body. Carrel [1] recognized the differences in the conditions under which cells proliferate within the animal and in tissue cultures, and made experiments in which he applied dead subcutaneous connective tissue to the wounds of dogs. He found that the cicatrization of the wounds was thereby delayed. He attributed the delay to the exclusion of external irritants that in turn stimulate the tissues themselves to produce substances hastening cell regeneration. Dvorak and Byram [2] applied various macerated tissues such as liver, kidney and spleen to wounds of the skin, but found no striking acceleration of healing. Both Carrel and Dvorak and Byram relied on macroscopic examination in a determination of the effects on the wounds. Previously Loeb and Spain [3] had recognized the limitations of the naked-eye appearances in a study of the healing of wounds. Since our purpose was to determine the effect of certain substances on specific cells, it was evident that a histologic study alone would suffice.

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1. Carrel, A.: Proc. Inst. Med., Chicago **8**:62, 1930.

2. Dvorak, H. J., and Byram, J. W.: Proc. Soc. Exper. Biol. & Med. **27**:967, 1930.

3. Loeb, L., and Spain, K. C.: J. Exper. Med. **23**:107, 1916.

PRODUCTION OF WOUNDS AND PREPARATION OF THE MATERIALS APPLIED TO THEM

The cell of the epidermis seemed well adapted for our study. It proliferates in its normal position in the skin and is a highly differentiated cell of specific type. Like other epithelial cells, the epidermoid cell maintains intimate contact with the vascularized membrana propria and, in the case of wounds, with the granulation tissue. Like the other epithelial cells, it thus derives its nutrition from the plasma and is brought under the regulatory mechanism that governs cell growth within the body.

The wounds were produced in young white rats weighing from 150 to 250 Gm. The rats were originally from the Wistar Institute and had been inbred in a more or less haphazard way for a number of years. The technic of producing the wounds was as follows: With the animal under ether anesthesia, the back of the neck was shaved and disinfected with tincture of iodine followed by alcohol. The loose skin was picked up by means of forceps, and two wounds of equal size and identical character were made by cutting through the fold of skin with a sharp 3 mm. leather punch. The two wounds were symmetrically located from 5 to 7 mm. from the roots of the ears. One of the wounds was filled with the macerated tissue or substance to be tested. Both wounds were covered with sterile gauze to a distance slightly beyond their margins. To prevent penetration of the collodion the gauze was impregnated wtih petrolatum. Dry cotton was spread over this and on the skin beyond. Celloidin was applied to the cotton. When dry, the neck was encircled by a neckband of stiff white cotton duck which was sewed in place. This was made snug, but yet not sufficiently tight to interfere with the circulation. Female rats in individual cages gave almost no trouble after the technic was learned.

The dead macerated tissue fell into two main groups. One was that of macerated epidermis. It was prepared in two ways. After the routine preparation and disinfection of the skin of the tail, the outer epidermal layers were removed by making strokes with a scalpel toward the root of the tail. Such scrapings included not only the cornified layers, but also a large percentage of deeper cells, including those from the rete malpighii. The scrapings were crushed in a mortar with a pestle and finally ground with a rotary motion. A few drops of sterile water were added, and the process was repeated. The mass was kept for twenty-four hours at room temperature and then in the icebox until used. The other preparation was made from 20 mm. embryos in a somewhat different way. The skin was removed from the body and spread on a sterile board with the dermis upward. Much of the myxomatous connective tissue was scraped away with a scalpel. The tissue including the epidermis was then macerated in the mortar.

The second group was that of macerated connective tissue, prepared from the tail tendons. The skin of the tail was slit and stripped from tip to root. The exposed tendons were pulled off and transferred to a sterile mortar. The tendons were first finely minced with scissors and then treated like the epidermis. The consistency was much the same as that of the original tendon. It was kept for twenty-four hours at room temperature before being placed in the icebox. The preparation of other macerated tissues was similar.

At the end of the experimental period, the gauze usually separated with ease from both the experimental and the control wound. In most instances, however, it was removed subsequent to fixation in 10 per cent formaldehyde. The wounds

and the surrounding tissue were removed, spread on blotter paper, to which they were fastened by pins, and then placed in formaldehyde. After fixation, the entire wound was cut into blocks; the blocks were embedded in paraffin and cut into serial sections of 10 microns' thickness. From the ribbon every fifteenth section was selected for staining in hematoxylin and eosin. Measurements were made by a calibrated micrometer ocular. The length of the epithelial tongue, which is the width of the regenerated epidermis, was taken at the center of the wound (middle block). The thicknesses in the tables are averages from the serial sections.

RESULTS OBTAINED WITH MACERATED EPITHELIUM

The successful healing of wounds of the skin is dependent on the proliferative activity of the two types of tissue present. Our investigation is concerned expecially with the epidermis. The epithelial tongues are the result of epithelial proliferation and were used as a measure of epidermal regeneration. The tongues extended downward to the base

TABLE 1.—*Experiments with Cornified Epithelium*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granulation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
91–A	3	0.672	1.844	0.264	0.0379	0.0180	0.0090
91–B	3	0.073	1.854	0.955	0.0028	0.0187	0.0075
92–A	3	0.382	2.096	0.300	0.0248	0.0222	0.0079
92–B	3	0.000	2.117	0.697	0.0000	0.0185	0.0075
108–A	5	1.270	2.664	0.372	0.0423	0.0415	0.0095
108–B	5	0.502	0.774	0.889	0.3978	0.0503	0.0089
109–A	5	1.422	2.680	0.692	0.0395	0.0318	0.0090
109–B	5	0.475	1.565	0.870	0.0375	0.0500	0.0075
110–A	5	0.900	3.108	1.032	0.0310	0.0330	0.0075
110–B	5	0.695	1.865	0.625	0.0187	0.0341	0.0075

* A = experimental wound; B = control wound.

of the wounds and then over the granulation tissue forming there. In this way the proliferating epithelium maintained contact with vascularized connective tissue affording the usual normal nutrition, and at the same time the proliferating cells were in direct contact with the solution products of the macerated epidermis. Both length and thickness of the regenerated epithelium in the wounds filled with macerated epidermis from the rat tail were much greater than in control wounds (table 1). In most instances the epidermal growth was such that it suggested the action of a special formative stimulus derived from the macerated epidermis. However, since the difference between the two wounds might be otherwise explained further experiments were made. That a mechanical effect was being exerted by the filling of the wounds appeared likely from the widths of those of longer duration (table 1). Wounds were therefore filled with a paraffin-petrolatum mixture of a consistency like that of the macerated tissues. These experiments showed the width of the wounds filled with the paraffin-petrolatum mixture to be greater

(table 2). The widths as recorded in the tables were distances between the lateral walls of the wounds. These widths did not correspond to the lengths of the epithelial tongues. Significant contrasts appear in a comparison of tables 1 and 2. In both series of experiments, the granulation tissue was quite active beneath the substances applied. In both, the epithelium extended down the sides and over the base, but beneath the macerated epidermis the extent of the epidermal growth was distinctly greater. Since our problem was concerned especially with the regeneration of the epithelium, a full consideration of all factors involved in

TABLE 2.—*Series of Experiments with Paraffin and Petrolatum Mixture*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granulation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
58-A	3	0.000	2.870	0.614	0.0000	0.0276	0.0100
58-B	3	0.020	1.464	0.772	0.0027	0.0260	0.0100
116-A	4	0.431	2.157	0.617	0.0284	0.0403	0.0100
116-B	4	0.270	0.744	0.564	0.0180	0.0360	0.0075
112-A	5	0.904	0.000	0.576	0.0335	0.0624	0.0100
112-B	5	0.422	0.000	0.424	0.0272	0.0324	0.0087

* A = experimental wound; B = control wound.

TABLE 3.—*Experiments with Fetal Epidermal Tissue*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granulation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
140-A	3	0.577	2.460	0.210	0.0477	0.0844	0.0100
140-B	3	0.079	2.447	0.527	0.0027	0.0211	0.0075
147-A	3	0.560	4.433	0.740	0.0292	0.0222	0.0075
147-B	3	0.079	2.447	0.527	0.0027	0.0211	0.0075
23-A	5	1.200	0.825	0.800	0.0566	0.1950	0.0125
23-B	5	0.215	0.430	0.600	0.0338	0.0744	0.0100

* A = experimental wound; B = control wound.

wound healing is unnecessary. Our examination of the normal control wounds confirms the observations of Akaiwa [4] in the rat that contraction plays an insignificant part, and that the healing is accomplished by the regeneration and movement of epithelium and by the formation of granulation tissue. Akaiwa further pointed out that the thickness of the stratum germinativum corresponds to the proliferative activity and energy of cell movements.

Confirmation of the stimulant action of macerated epidermis on epidermal cells was sought by employing fetal epidermis, which was prepared in the manner previouly described. Again the epidermis was much more active than that of the control wounds (table 3). This

4. Akaiwa, H.: J. M. Research **40**:371, 1919.

preparation stimulated not only the epidermis, but also the production of granulation tissue, which was the greatest seen in the entire series of experiments. In this connection it is significant that the preparation contained considerable myxomatous dermis and subcutaneous tissue, while the scrapings from the adult tail contained little connective tissue.

A commercial keratin (Merck) was brought to the consistency of the macerated tissue and applied to the wounds (table 4). The length of the epithelial tongue and the width of the defect in the wounds to which the keratin was applied indicate that it was without effect or that it

TABLE 4.—*Experiments with Commercial Keratin*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granulation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
126-A	4	0.270	4.200	0.520	0.0337	0.0225	0.0075
126-B	4	0.347	4.080	0.450	0.0286	0.0250	0.0075
125-A	5	0.250	3.786	1.850	0.0159	0.0194	0.0100
125-B	5	0.400	0.000	0.258	0.0242	0.0200	0.0083

* A = experimental wound; B = control wound.

TABLE 5.—*Experiments with Ether and Alcohol Treated Cornified Epidermis*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granulation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
128-A	4	0.203	2.935	0.300	0.0172	0.0158	0.0075
128-B	4	0.417	0.833	0.618	0.0201	0.0314	0.0075
127-A	5	0.205	3.195	0.550	0.0144	0.0212	0.0100
127-B	5	0.463	0.460	0.633	0.0237	0.0279	0.0100

* A = experimental wound; B = control wound.

retarded epidermization somewhat. Since the source and composition of this product were unknown, the results obtained are not offered as proof that rat keratin would be without effect.

Alcohol-Ether Treated Epidermis.—Scrapings from the rat tail were macerated in the usual way and then ground with absolute alcohol, which was decanted, and the operation twice repeated. The residue was then treated with ether in the same fashion. The product was a whitish powder, which was brought to the desired consistency by the addition of a few drops of sterile distilled water. Two obvious changes were brought about, which consisted of the removal of lipoids and the coagulation of protein. Epidermal regeneration in this series (table 5) was not increased. The length and the thickness of the tongues were less than in the control wounds.

One attempt was made to extend the experiments to an unrelated epithelial tissue, the liver. It was crushed after washing out the blood while in situ and applied in the usual way. The proliferative activity of the epidermis was not much affected. The length of the tongues was somewhat greater than in the control wounds, but their thickness was variable (table 6).

RESULTS OBTAINED WITH MACERATED CONNECTIVE TISSUE

As in the preceding experiments, with macerated connective tissue the cells of the stratum germinativum adjacent to the wound increased

TABLE 6.—*Experiments with Liver Tissue*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granulation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
137-A	3	0.380	2.853	0.327	0.0225	0.0437	0.0075
137-B	3	0.337	2.423	0.163	0.0150	0.0533	0.0075
138-A	4	0.523	0.523	0.600	0.0316	0.0304	0.0083
138-B	4	0.315	2.620	0.090	0.0309	0.0325	0.0088

* A = experimental wound; B = control wound.

TABLE 7.—*Experiments with Connective Tissue*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granulation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
42-A	3	0.010	3.836	0	0.0026	0.0179	0.0075
42-B	3	0.000	2.306	0.424	0.0000	0.0088	0.0075
53-A	3	0.172	3.100	0.282	0.0240	0.0481	0.0102
53-B	3	0.010	2.416	0.764	0.0030	0.0227	0.0100
102-A	4	0.200	2.280	0.263	0.0185	0.0285	0.0079
102-B	4	0.417	0.833	0.613	0.0201	0.0314	0.0075
104-A	4	0.073	2.520	0.790	0.0146	0.0241	0.0095
104-B	4	0.417	0.833	0.613	0.0201	0.0314	0.0075
96-A	5	0.174	3.666	0.456	0.0076	0.0496	0.0100
96-B	5	0.840	0.700	0.860	0.0147	0.0284	0.0100
97-A	5	0.183	3.208	0.688	0.0100	0.0320	0.0075
97-B	5	0.510	0.000	1.050	0.0100	0.0354	0.0100

* A = experimental wound; B = control wound.

in size and moved toward the margin of the wound soon after its production. These changes and the increase in mitoses contributed to the thickening and extension of the epithelium. In the control wounds, the average thickness of the basal layer was 0.0211 mm. on the third day, 0.0314 mm. on the fourth and 0.0377 mm. on the fifth, while the normal thickness was about 0.0075 (table 7). The increase in the length of the epithelial tongues on which epidermization of the wound depends started on the fourth day in both experimental and control wounds. In the control wounds there was a progressive daily increase, while in the experimental ones there was much individual variation. In all, the

epithelium showed a tendency to extend over the surface of the dead tissue rather than beneath it. After the fourth day the lagging of epidermization in these experiments was evident. We were able therefore to confirm the observations of Carrel,[1] who covered the wounds with dead subcutaneous connective tissue.

COMMENT

Dead macerated epidermis, both adult and fetal, promotes the proliferation of the epidermal cells. The stimulant action is local, since control wounds only 2 cm. distant do not show it. That the increase in growth is not mechanical and due to a protective influence of an inert substance is shown by its absence in the wounds filled with the paraffin-petrolatum mixture. The evidence is that from the macerated epidermis undergoing local disintegration there is liberated a formative stimulus which, so far as one may judge from our experiments, is specific for the epidermal cells. The soft macerated epidermis physically offers little resistance to the extension of cells into it, and yet the epidermis extends beneath it. This behavior is seen also in the paraffin-petrolatum wounds and no doubt is brought about by the concentration of nutrition in this situation. Although the macerated epidermis stimulates epidermal regeneration, it appears not to afford complete nutriment to the proliferation of epidermoid cells. The growth promoting substance is not present in other macerated tissues, and it is destroyed by alcohol ether extraction. Baker and Carrel [5] found that the addition of primary derivatives of protein to the media accelerated the growth of cultures of guinea-pig skin, and Carrel [1] mentioned that these substances are normally present in organs, and that they may be manufactured by leukocytic ferments from cell débris and coagulated proteins. Hammett [6] advanced the hypothesis that relatively simple substances containing the sulphydril group, such as glutathione, excite cells to proliferate. Voegtlin and Chalkley [7] demonstrated the stimulant action of this tripeptide on Amoeba proteus. Girand and Bulliard [8] found cornified epidermis to be relatively poor in glutathione, while the liver is rich in this substance. So far as we can determine, our results give little information in regard to the part of the epithelial cell that acts as the stimulant, but they tend to show that the stimulus for the epidermoid cell is peculiar to the macerated epidermis and is not of a general nature as are the polypeptide derivatives of fibrin.

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5. Carrel, A., and Baker, L. E.: J. Exper. Med. **46**:503, 1926. Baker, L. E., and Carrel, A.: ibid. **47**:353, 1928.

6. Hammett, F. S.: Protoplasma **7**:20, 1929.

7. Voegtlin, C., and Chalkley, H. W.: Pub. Health Rep. **45**:30, 1930.

8. Girand, A., and Bulliard, H.: Compt. rend. Soc. de biol. **98**:500, 1928.

We found, as did Carrel, that the healing of a wound is retarded by filling it with macerated connective tissue. The histologic study of our wounds revealed a tendency of the epidermis to extend over this dead tissue to a point where lack of nutrition halted the growth. In several instances, the dead tissue, more tenacious than the other macerated tissues used, offered mechanical obstruction to the extension of the epidermis in any other direction. We are therefore inclined to favor the view that the delay in cicatrization was due to mechanical and nutritional rather than to chemical factors. A close study was made of these wounds to determine whether the macerated tendon might stimulate fibroblastic proliferation, but no consistent evidence of this was obtained. On the other hand, the fetal skin that contained immature connective tissue unquestionably stimulated the formation of angiofibroblastic tissue and produced the most abundant granulation tissue seen.

CONCLUSIONS

Macerated homologous epidermis applied to wounds stimulates epidermal regeneration.

Evidence was obtained which indicates that the stimulation is a chemical action and not the indirect result of a change in physical environment.

Failure of other macerated tissues to act similarly is suggestive of the presence in the epidermis of a growth-promoting substance specific for the epidermoid cell.

General Review

AN ASPECT OF INFLAMMATION IN RELATION TO IMMUNITY *

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The experimental studies on inflammation began about seventy years ago, when Cohnheim [1] demonstrated the migration of leukocytes from the blood into the tissue spaces. This, according to Cohnheim, was primarily the result of injury to the walls of the capillaries with the consequent passive passage of plasma constituents and blood corpuscles into the tissue spaces. Metchnikoff [2] regarded inflammation as a phagocytic reaction to injurious material that had penetrated the tissues. The leukocytes either came from the blood or were modified cells of the tissues at the site of the injury:

L'inflammation doit donc être envisagée dans son ensemble comme une réaction phagocytaire de l'organisme contre les agents irritatifs, réaction qui tantôt s'accomplit par les phagocytes mobiles seuls, tantôt avec le concour des phagocytes vasculaires ou celui du système nerveux. (One has to imagine inflammation as a phagocytic reaction of the organism against the irritating agents, a reaction which sometimes is accomplished by the mobile phagocytes alone and sometimes with the aid of the vascular phagocytes or those of the nervous system.)

Inflammation was not regarded by Metchnikoff as primarily a lesion of the vascular walls in the sense of Cohnheim, but rather as an adaptive reaction on the part of phagocytic cells for the express purpose of protecting the organism as a whole. I need not dwell on these early views, except to note that the migration of leukocytes at the point of injury was established as the essential phase of the inflammatory reaction. Other elements of the reaction, such as would accompany an outpouring of plasma into the tissue spaces, were more or less relegated to the background. Some of the early writers, however, pointed out the importance of deposits of fibrin in certain types of inflammation. The observations of Adami [3] are significant in this connection:

Even when inflammation (as in pericarditis) affects the whole extent of a serous cavity, the layer of fibrin acts as a protective coat closing the lymphatic

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* From the Department of Pathology, Harvard Medical School.

1. Cohnheim, J.: Virchows Arch. f. path. Anat. **40**:1, 1867; Vorlesungen, ed. 2, Leipzig, 1882.

2. Metchnikoff, E.: Leçons sur la pathologie comparée de l'inflammation, Paris, G. Masson, 1892.

3. Adami, G. J.: Inflammation, London, The Macmillan Company, 1909.

"stomata" hindering the free absorption of the morbid material by the lymph and blood vessels, and filtering bacteria out of such fluid as does find its way through to the tissues beneath.

Opie [4] defined inflammation as a process by means of which cells and serum accumulate about an injurious substance and tend to remove or destroy it.

In the usual textbook description of the inflammatory reaction, except for an occasional reference to the importance of the accumulation of serum at the site of inflammation, the predominant rôle of the leukocytes completely overshadows the activities of all other elements.

In the last few years evidence has been brought forward to show that the inflammatory reaction plays a definite rôle in localizing the irritating factor, or at least limiting its dissemination, whether it is a bacterial or a chemical irritant. This capacity, which may appropriately be termed fixation, contributes a further significance to inflammation in relation to immunity. By delaying the dissemination of obnoxious substances into the circulating blood stream, the nonspecific as well as the specific inflammatory reaction protects the organism as a whole at the expense of local injury. This view of inflammation has gained considerable ground in the last few years. Recent analyses have demonstrated that fixation at the site of inflammation is due to mechanical obstruction caused by a network of fibrin and to occlusion of lymphatic vessels.[4a] Studies on the time relationship have shown that fixation is the initial phase in the development of the inflammatory reaction, even preceding the migration of leukocytes. This is doubtless due to injury of the capillaries causing an increase in the permeability of their walls, which results in an outpouring of fibrinogen into the tissue spaces. Pathologists are gradually reverting to the concept of Cohnheim, namely, that in inflammation the primary injury is to the wall of the capillaries. Furthermore, the early development of thrombi in the lumen of lymphatics is doubtless the result of severe initial injury to the lymphatic wall itself in an inflamed area. In view of present evidences, as will be pointed out, one can ascribe a definite function to the early formation of fibrin at the site of inflammation. Fixation plays a definite rôle in inflammation and may be considered in the same light as the migration of leukocytes and the stage of fibrous repair that follow. Chronologically the inflammatory reaction may be broadly divided, therefore, into three phases, which, it is understood, may overlap: (1) fixation, (2) emigration of leukocytes and (3) repair.

The last two phases are well known to pathologists and will not be dwelt on further. The purpose of this review is to describe the evidences that recently have accumulated which show that with the initial increase

4. Opie, E. L.: Arch. Int. Med. **5**:541, 1910; J. Immunol. **17**:329, 1929.

4a. Menkin, V.: J. Exper. Med. **53**:171 and 179, 1931; Arch. Int. Med., in press.

in the permeability of the capillaries and the passage of plasma constituents into tissue spaces the inflammatory reaction is capable of rapidly circumscribing the irritant and thus allowing a definite interval of time for the leukocytes to assemble for phagocytosis. Fixation, as the initial phase of the inflammatory reaction, therefore becomes of distinct importance as a mechanism in immunity.

FIXATION OF BACTERIA BY THE NONSPECIFIC INFLAMMATORY REACTION

The dissemination of foreign substances from the site of injection into the lymphatics and into the blood stream has been studied by numerous investigators. Muscatello [5] showed that carmine and various other inert particles, when injected into the peritoneal cavity, reached the anterior mediastinal lymph nodes very rapidly. Noetzel [6] injected bacteria (*Bacillus pyocyaneus*) into the knee joints of rabbits; from five to ten minutes later he was able to demonstrate the presence of the organisms in the inguinal, crural and lumbar lymph glands. Buxton [7] found that typhoid bacilli, within a few minutes after their injection into the peritoneal cavity, appeared in great numbers in the blood stream. Wells and Johnstone [8] showed that the absorption of bacteria from the peritoneal cavity takes place through lymphatic vessels.

Does the inflammatory reaction retard the dissemination of micro-organisms from the site of inflammation? The studies of Issayeff [9] are interesting in this connection. He showed that the peritonitis induced by a variety of sterile irritants, such as foreign blood serum, bouillon or normal salt solution, temporarily increased resistance to subsequent intraperitoneal injection of bacteria. Pawlowsky [10] repeated the observations of Noetzel and demonstrated the presence of staphylococci in the blood and organs of guinea-pigs from twenty-four to forty-eight hours after inoculation of the knee joint. If before inoculation an acute inflammation of the knee joint had been produced by the injection of a sterile irritant, such as turpentine, alcohol or quinine solution, the dissemination of the micro-organisms was either inhibited or wholly prevented.

Rivers and Tillett [11] confirmed these studies by finding that beef broth injected into the skin of rabbits protected against streptococci inoculated twenty-four hours later. No retardation was observed when

5. Muscatello, G.: Virchows Arch. f. path. Anat. **142**:327, 1895.
6. Noetzel, W.: Beitr. z. klin. Chir. **51**:740, 1906.
7. Buxton, B. H.: J. M. Research **16**:17, 1907.
8. Wells, H. G., and Johnstone, O. P.: J. Infect. Dis. **4**:582, 1907.
9. Issayeff: Ztschr. f. Hyg. u. Infectionskr. **16**:287, 1894.
10. Pawlowsky, A. D.: Ztschr. f. Hyg. u. Infectionskr. **62**:433, 1909.
11. Rivers, T. M., and Tillett, W. S.: J. Exper. Med. **41**:185, 1925.

the micro-organisms were inoculated simultaneously with the beef broth. Mallory and Marble [12] concluded that the local resistance produced by filtrates of staphylococci was due to local inflammation and was therefore not specific. It is to be recalled that Besredka [13] advocated the therapeutic use of such filtrates. Opie [14] showed that acute inflammation of the peritoneal cavity caused by aleuronat retarded the rush of injected hemolytic streptococci from the peritoneal cavity into the circulating blood and after twenty-four hours completely prevented it. By a culture method I [15] demonstrated that *B. prodigiosus* injected into an inflamed area was fixed in situ and failed to disseminate into the tributary lymphatics.

Some authors believe that in inflammation the large mononuclear phagocytes are responsible for protection. Nakahara [16] injected olive oil into the peritoneal cavity and showed that after forty-eight hours an exudate was formed containing from 65 to 85 per cent macrophages. At this stage the resistance of mice to *B. coli* introduced into the peritoneal cavity was definitely increased. Gay and Morrison [17] studied the changes in resistance to intrapleural inoculation of streptococci after a sterile inflammation had been previously caused by various irritants. They contended that the increased resistance to the pathogenic microorganism resulted from the accumulation of clasmatocytes. This interpretation was questioned by Opie,[14] who maintained that no data had been offered that determined the rôles of polymorphonuclear leukocytes, mononuclear cells, serum or indeed increased permeability of blood vessels in overcoming the injurious effects of sterile irritants or of bacteria. Gay and Clark [18] in answer to these criticisms produced some evidence showing that perhaps the polymorphonuclear leukocytes were relatively inactive in increasing the resistance of their animals. However, the increased resistance to virulent bacteria brought about by previously "preparing" a cavity with a sterile irritant is evident only when the inflammatory reaction has been going on in the cavity for from forty-eight to seventy-two hours. Local fixation of substances at the site of inflammation, as will presently be shown, can be demonstrated a relatively short time (sometimes after only thirty minutes) after the introduction of the inflammatory irritant.[19] For this reason the increased resistance to intrapleural inoculation of streptococci found by Gay and

12. Mallory, T. B., and Marble, A.: J. Exper. Med. **42**:465, 1925.
13. Besredka, A.: Immunisation locale, Paris, Masson & Cie, 1925.
14. Opie (footnote 4, second reference).
15. Menkin, V.: J. Exper. Med. **53**:647, 1931.
16. Nakahara, W.: J. Exper. Med. **42**:201, 1925.
17. Gay, F. P., and Morrison, L. F.: J. Infect. Dis. **33**:338, 1923.
18. Gay, F. P., and Clark, A. R.: Proc. Soc. Exper. Biol. & Med. **27**:995, 1930.
19. Menkin, V.: J. Exper. Med. **50**:171, 1929.

his collaborators probably has nothing to do with the initial phase of local fixation in inflammation, but is evidently referable to a later phase of the inflammatory reaction.

FIXATION OF BACTERIA BY THE SPECIFIC INFLAMMATORY REACTION

Koch [20] described the immune reaction that takes place when a tuberculous guinea-pig is reinoculated with either living or dead tubercle bacilli. This reaction to reinfection is known as the Koch phenomenon and forms probably the most important single principle in the understanding of immunity in tuberculosis. A part of Koch's original description follows (cited from a translation by Pottenger [21]):

> When one inoculates healthy guinea-pigs with a pure culture of tubercle bacilli the inoculation wound closes up and apparently heals within a few days. In the course of ten to fourteen days, however, a hard nodule appears, which breaks down and forms an ulcer. This remains until the death of the animal. It is quite different, however, when a guinea-pig that is already tuberculous is inoculated. Animals that have been inoculated from four to six weeks previously are best suited to this purpose. In one such animal, however, the inoculation did not heal in the beginning, but small nodules appeared, and within one or two days showed characteristic changes about the point of inoculation. Around the point of inoculation it became hard and took upon itself a darker color, which did not confine itself to the immediate point of inoculation but extended to the surrounding tissues for a distance of 0.5 to 1 cm. When the succeeding dose is administered it shows very distinctly that the skin showing the alteration is necrotic; and it is eventually cast off, a small superficial ulceration remaining, which usually heals quickly and permanently without the regional lymph-glands becoming involved. . . .
>
> After these characteristic effects had been discovered, I followed them in every direction and I found that pure cultures of tubercle bacilli that had been killed, ground up, and mixed with water could be injected in large amounts subcutaneously in healthy guinea-pigs without producing anything beyond a local suppuration. Tuberculous guinea-pigs, on the contrary, were killed by the injection of very small amounts of such cultures of dead bacilli within six to forty-eight hours after administration of the dose. A dose that is not sufficiently large to kill an animal often produces an extensive necrosis of the skin in the neighborhood of the site of injection.

Pirquet called the altered response of tuberculous tissue to reinfection "allergy." The reaction to tuberculin, which may be regarded as a manifestation of the Koch phenomenon by means of tuberculoprotein, is histologically an acute inflammation, as pointed out by Opie.[14] There is an abundant accumulation of serum and polymorphonuclear leukocytes during the first forty-eight hours. Polymorphonuclear leukocytes are finally displaced by mononuclear cells, partly at least as the result of phagocytosis.

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20. Koch, R.: Deutsche med. Wchnschr. **17**:101, 1891.
21. Pottenger, F. M.: Tubercle **10**:409, 1929.

In 1916, Kraus [22] mentioned how the work of Pawlowsky and Issayeff cited by Opie (1910)[4] had suggested to him the possibility that specific immunity to reinfection in tuberculosis might be a function of allergy in that the inflammation accompanying the latter prevents the spread of reinfecting bacilli. Later, Kraus [23] put the case more concretely as follows: "The tuberculous animal responds with exaggerated or overreaction to irritation and throws up a barrier against the bacilli much more actively than normally and before the bacilli can get under way."

Kraus and Willis [24] showed experimentally the extent of the retardation in infected animals. Willis [25] studied the dissemination of tubercle bacilli from the site of cutaneous inoculation, and observed that in reinfected guinea-pigs the spread of tubercle bacilli from the site of inoculation was retarded, whereas in normal animals the organisms passed readily to the regional lymph nodes. Both Kraus and Willis laid emphasis on the mechanical barrier set up against the outward movement of the deposited bacilli. "We believe, therefore, that specific tuberculo-immunity occurs through a fixation of germs that results from the operation of the allergic reaction. An almost immediate inflammatory outpouring hems in the bacilli more or less effectively and thus delays or prevents their spread, which is so facile and rapid in the non-tuberculous, non-allergic animal." [26]

Rich and McCordock [27] attacked the hypothesis of Kraus and Willis, largely because of certain apparent inconsistencies in the arguments of the latter. In the first place, Rich and McCordock pointed out that, although it was assumed that inflammation per se can limit the immediate spread of micro-organisms, there was not the slightest proof of this. "And until we have more precise information on the question it must be remembered that the inoculation results in the experiments of Kraus and Willis might be interpreted on the basis of the well established fact that there occurs a much greater destruction of bacilli in immune animals than in normal ones." An interpretation of this sort would be difficult to accept in view of the facts obtained. Willis [25] demonstrated that the regional lymph nodes in immune animals were infected at the end of two weeks after cutaneous inoculation. If the apparent retardation of bacilli at the site of inoculation was really due to their destruction in situ by a subtle immunologic mechanism, their

22. Kraus, A. K.: Tr. Nat. A. Prev. Tuberc. **12**:253, 1916.
23. Kraus, A. K.: Am. Rev. Tuberc. **1**:65, 1917.
24. Kraus, A. K., and Willis, H. S.: Tr. Nat. A. Prev. Tuberc. **20**:277, 1924.
25. Willis, H. S.: Am. Rev. Tuberc. **11**:427 and 439, 1925.
26. Kraus, A. K.: Am. Rev. Tuberc. **11**:343, 1925.
27. Rich, A. R., and McCordock, H. A.: Bull. Johns Hopkins Hosp. **44**:273, 1929.

appearance after a latent interval of two weeks in the regional lymph nodes would be difficult to explain. Furthermore, I [15] recently demonstrated that an acute inflammatory reaction caused by a nonspecific irritant prevented for at least several hours the dissemination of *B. prodigiosus* to the tributary lymph nodes. A distinctly larger number of micro-organisms were recovered from the site of inflammation than from the corresponding normal control area. These experiments demonstrated that an acute inflammation per se retards the dissemination of bacteria into the regional lymphatics.

Kraus and Willis [28] showed that reinfection with tubercle bacilli in areas where an allergic inflammation had been induced by tuberculin caused more widespread tuberculosis than that seen in reinfected control animals that had received no tuberculin. Their observations have been brought forward as evidence that the allergic inflammation does not retard the dissemination of bacilli. Such an interpretation of these particular experiments, however, is open to some doubt. It is possible, in view of recent observations that I made [29] on the accumulation of foreign proteins in inflamed areas, that preliminary treatment of infected animals with tuberculin might cause an accumulation from the blood stream of tuberculoprotein in various inflamed tuberculous lesions of the body, which by its presence there would intensify the preexisting local inflammatory reaction.

The view that resistance may be at times independent of allergy and thus be referable entirely to a subtle immunologic mechanism is not questioned. Zinsser and his collaborators [30] expressed the view that "the fundamental biological significance of bacterial allergy is an increased specific adjustment of the tissues for response to the stimulus of infection, and a consequently enhanced capacity for the rapid mobilization of a protective mechanism." The fundamental point, however, is this: When an allergic inflammation develops as a result of reinfection, does the inflammatory reaction play a definite rôle in the mechanism of immunity by retarding the dissemination of bacteria? Evidences cited and some that are to follow confirm the view that an acute inflammation either of specific origin or caused by a nonspecific irritant definitely delays the spread of injurious substances from the site of inflammation.

Wadsworth [31] injected virulent pneumococci into the tracheas of normal animals and thereby caused fatal bacteremia which in some cases was associated with bronchopneumonia. When immunized animals were

28. Kraus, A. K., and Willis, H. S.: Am. Rev. Tuberc. **4**:563, 1920.

29. Menkin, V.: J. Exper. Med. **52**:201, 1930.

30. Zinsser, H.; Ward, H. K., and Jenning, F. B., Jr.: J. Immunol. **10**:719, 1925.

31. Wadsworth, A.: Am. J. M. Sc. **127**:851, 1904.

treated in the same manner, there was a diffuse exudation comparable with lobar pneumonia, but no invasion of the blood stream. Cecil and Blake [32] also found that the incidence of invasion of the blood stream was less in monkeys vaccinated against pneumonia induced by intratracheal inoculation than in nonvaccinated animals. Cannon and Pacheco [33] described histologic studies of the skin and subcutaneous tissues of the abdominal walls of normal guinea-pigs and of animals previously immunized by intracutaneous injections of a staphylococcal vaccine. All the animals were infected by the intracutaneous injection of a live virulent culture of *Staphylococcus aureus*. In the normal animals the staphylococci disseminated throughout the subcutaneous tissues as a cellulitis. In previously immunized animals, on the other hand, the staphylococci tended to remain localized near the site of inoculation. These investigators expressed the belief that the immunity secured was predominantly cellular, with the tissue macrophages playing the dominant part. Localization of the micro-organisms was also reinforced to some extent by the action of agglutinating or of opsonizing antibodies.

FIXATION OF FOREIGN PROTEINS BY THE SPECIFIC INFLAMMATORY REACTION

Evidence has been cited showing that immunization retards the dissemination of bacteria from the site of injection, where specific inflammation occurs. Extensive studies by Opie [34] demonstrated that a foreign protein injected into the skin of a sensitized animal is fixed at the site of injection. This fixation is accompanied by an acute inflammatory reaction (Arthus' phenomenon).

When a small quantity of a foreign protein, such as horse serum or egg white, is injected into a normal rabbit, it is demonstrable by the precipitin reaction in the blood of that animal for a period of from seven to nine days. Opie [35] showed that with continued immunization injected proteins exhibit a decreasing tendency to find their way into the blood. After a few immunizing injections they can be found in the blood serum in only small quantities and for but a short time. After the sixth or seventh injection well immunized animals reveal no trace of the proteins in the circulating blood.

Opie undertook to determine what happened to a foreign protein at the site of injection in the normal and in the immune animal. The cutaneous site of injection was excised, extracted with normal salt solution and tested by the precipitin reaction for the presence of the foreign

32. Cecil, R. L., and Blake, F. S.: J. Exper. Med. **31**:519, 1920.

33. Cannon, P. R., and Pacheco, G. A.: Am. J. Path. **6**:749, 1930.

34. Opie, E. L.: J. Immunol. **9**:231, 1924; J. Exper. Med. **39**:659, 1924; footnote 4, second reference.

35. Opie (footnote 34, second reference).

protein. The concentration of the foreign protein extracted was estimated by comparison with solutions of known protein content. Crystalline egg albumin injected into a normal rabbit was found in small concentration on the first and second day (1:250 and 1:500, respectively). On the third day it had disappeared from the site of injection. In an immunized animal, on the other hand, the concentration of the protein was much greater at the site of injection than in a corresponding normal area. In one experiment the foreign protein was recovered on the first day in a concentration of 1:80 at the periphery of the inflamed area; on the second day in a concentration of 1:50; it did not disappear until the fifth day.

In this way Opie demonstrated that in the immunized animal, fixation of the antigen at the site of injection prevented its dissemination throughout the body. Opie [36] expressed the opinion that specific precipitation had a part in this fixation. The precipitate formed by the contact of antigen and antibody has a strong attraction for polymorphonuclear leukocytes of the blood and, according to Opie, is doubtless digested and destroyed by these cells. In a later study in Professor Opie's laboratory I [29] demonstrated that a nonspecific acute inflammatory reaction is capable of arresting a foreign protein at the site of injection. For this reason it is probable that the fixation of antigen in Arthus' phenomenon is largely due to the acute inflammatory reaction brought about by the contact of antigen and antibody in the tissues. Opie [36] pointed out that it is unnecessary to assume that the tissues have undergone any essential alteration as the result of sensitization. "Acute inflammation in response to a specific irritant, namely, Arthus' phenomenon, occurs when the antigen concerned and its antibody meet within the tissues." This conclusion was reached by reversing the usual procedure. A normal animal received 10 cc. of horse serum intravenously. On the following day 0.5 cc. of strong anti-horse serum from an immunized rabbit was injected into the skin. Extensive inflammatory edema (Arthus' phenomenon) occurred at the site of injection, whereas none followed the injection of normal rabbit serum. Opie viewed the anaphylactic inflammation in which the antigen is fixed at the point of entry as an apparent paradox between susceptibility to injury and the resistance of immunity. With the anaphylactic inflammation the vital organs are protected at the expense of local injury. In a recent review Nordmann [37] criticized Opie's interpretation of Arthus' phenomenon. This author maintained that ordinary inflammatory phenomena such as are experimentally produced by the simultaneous injection of two serums into a normal animal do not show the gradually increasing severity of

36. Opie, E. L.: Tubercle **7**:23, 1925.
37. Nordmann, M.: Physiol. Rev. **11**:41, 1931.

symptoms that is the characteristic feature of the Arthus' phenomenon. It is clear, however, that this gradual change in severity of symptoms in anaphylactic inflammation may be to some extent (though not entirely) correlated with the increase in precipitin during the process of immunization. Nordmann insisted that physiologic alterations play an important rôle in the development of Arthus' phenomenon: "We conclude therefore that the cause of the final necrosis following the later injections in the Arthus experiment is due to the ability of the arteries to become accustomed to the serum and that in consequence of this adaptation they are less and less altered by additional injections of the antigen." Although it is perfectly natural that physiologic changes should accompany the anaphylactic inflammation, it is doubtful whether this author has demonstrated that the particular changes in the arteries influence the development of the anaphylactic inflammatory reaction. The studies of Opie indicate that this reaction is brought about by the contact of antigen and antibody at the site of injection. An inflammatory reaction of unusual intensity occurs, and this reaction doubtless brings to the site of inflammation plasma containing more precipitin. This view is in agreement with facts recently demonstrated by myself,[29] namely, that foreign proteins from the circulating blood stream accumulate in an inflamed area. The initial anaphylactic inflammation is intensified, and this may account for the gradual increase in the severity of the symptoms of Arthus' phenomenon.

ACCUMULATION AND FIXATION OF FOREIGN SUBSTANCES AT SITE OF INFLAMMATION

In an inflamed area the normal functional equilibrium between cells, intercellular fluids and blood is doubtless profoundly modified. Local physiologic changes are involved, affecting the permeability of the capillaries, the rate of the blood flow and the balance of the body fluids. The increase in the permeability of the capillaries is shown by the local edema. The proteins of the plasma to a varying extent pass out into the tissue spaces, disturbing, doubtless, the osmotic relationship between the fluids of the tissues and the plasma of the blood. About thirty years ago Adler and Meltzer[38] concluded that the passage of fluids from tissue spaces into lymphatics probably depended on osmosis, perhaps assisted to some degree by filtration. Starling[39] pointed out that capillary filtration was the resultant of two forces: capillary pressure, on the one hand, and the osmotic pressure exerted by the plasma colloids, on the other. Recently this conception has been confirmed experimentally by the work of Landis,[40] who showed by measuring directly intracapil-

38. Adler, I., and Meltzer, S. J.: J. Exper. Med. **1**:482, 1896.
39. Starling, E. H.: J. Physiol. **19**:312, 1895-1896.
40. Landis, E. M.: Am. J. Physiol. **81**:124, 1927.

lary pressure that the rate of filtration depends on the difference between intracapillary pressure and the osmotic pressure of the plasma colloids. By this direct method he found that whereas filtration occurs at the arterial ends of capillaries, absorption from tissue spaces takes place at the venous ends. Churchill, Nakazawa and Drinker,[41] however, demonstrated that, in the frog, at least, the osmotic pressure of the plasma proteins is lower than indicated by the figures given by White [42] and used by Landis in his interpretations. For this reason these investigators thought it safer to conclude that there exists a filtration of fluid throughout the system. Rous and his collaborators [43] cast some doubt on the results obtained by Landis. They showed that a vital dye injected intravenously diffuses first into the tissue spaces on the venous ends of the capillaries. These investigators maintained that there is a mounting gradient of permeability throughout the length of the capillary. This was demonstrated in a variety of organs in different animals. Furthermore, when they made the same observations as Landis, with only slight modifications, on injection of a vital dye into the vessels of the mesentery of the frog, they were unable to confirm his findings of increased filtration of dye at the arterial ends of the capillaries.

The normal equilibrium of the filtration through the wall of the capillary, however, is doubtless modified in inflammation by the passage of proteins from the plasma into the tissue spaces. If the passage of lymph from tissue spaces into afferent lymphatic vessels depends largely on an osmotic equilibrium, it is probable that the accumulation of plasma proteins in the tissue spaces modifies and retards the flow of lymph into the afferent lymphatic vessels. Schade and Menschel [44] found that in inflamed areas, especially in those with suppuration, the accumulation of products of tissue degeneration may become so great that the osmotic pressure is raised as high as 11 atmospheres. An inflamed area can be considered as shunted off from the rest of the organism. It has its own metabolism, its own hydrogen ion concentration, and, as was just pointed out, its own modified circulation.

I studied the behavior of foreign substances injected into an inflamed area by direct observation of the tributary lymphatic nodes and vessels draining the area. After some preliminary experimentation with the nucleated corpuscles of fowls, a vital dye, trypan blue, was tried and found to be satisfactory.[10] If trypan blue is injected into the normal

41. Churchill, E. D.; Nakazawa, F., and Drinker, C. K.: J. Physiol. **63**:304, 1927.

42. White, H. L.: Am. J. Physiol. **68**:523, 1924.

43. Rous, P.; Gilding, H. P., and Smith, F.: J. Exper. Med. **51**:807, 1930. Smith, F., and Rous, P.: J. Exper. Med. **53**:195, 1931. Rous. P., and Smith, F.: J. Exper. Med. **53**:219, 1931.

44. Schade, H., and Menschel, H.: Ztschr. f. klin. Med. **96**:279, 1923.

subcutaneous tissue of the foreleg of a rabbit, within from twenty to thirty minutes the dye stains the tributary afferent lymphatic vessels, the lymph node and the efferent lymphatic vessel. If, on the other hand, the dye is injected into a similar area in which an inflammatory reaction has been produced some time before by a sterile irritant, the dye does not appear in the tributary lymphatics; the afferent lymphatic vessels, the lymph node and the efferent lymphatic vessel remain colorless. The dye is evidently retained in the inflamed area. The fixation of the dye takes place soon after the injection of the irritant, being observed in some instances when the dye is injected thirty minutes after the injection of the irritant.

Since trypan blue tends to remain in situ when injected directly into the inflamed area, an attempt was made, at the suggestion of Prof. Eugene L. Opie, to determine whether dye injected into the blood stream would accumulate at the site of inflammation. MacCurdy and Evans [45] pointed out that the normal brain and spinal cord remain free from dye injected intravenously, but that areas of damage, such as softening or inflammation, become deeply stained. Bowman, Winternitz and Evans [46] found that trypan blue injected intravenously stained tubercles in experimental tuberculosis. Subsequently Winternitz and Hirschfelder [47] demonstrated that this dye injected in experimental lobar pneumonia stained the consolidated area of the lung selectively: "The intravenous injection of trypan blue and trypan red gave rise to the usual diffuse staining as described by Bouffard, Goldman, etc., but in addition to this the diseased area of the lung showed a much more intense staining than any of the other tissues, while the normal lung tissue was practically normal in color." Lewis [48] found that when the cornea of a rabbit was inoculated with a living culture of the tubercle bacillus, a progressive lesion resulted characterized by intense congestion of the conjunctiva. When the animal received an intravenous injection of trypan red twenty-four hours or more after such an inoculation, the fluid in the anterior chamber of the inoculated eye became colored. Precisely similar results were obtained when abrin was administered in the conjunctiva as an inflammatory irritant. McClellan and Goodpasture [49] showed that trypan blue accumulated in lesions of herpetic encephalitis in the rabbit's brain, the injured areas presenting a striking color against the unstained healthy tissue of the brain. Siengalewicz [50] found that general damage to the

45. MacCurdy, I. T., and Evans, H. M.: Berl. klin. Wchnschr. **49**:1695, 1912.
46. Bowman, F. B.; Winternitz, M. C., and Evans, H. M.: Centralbl. Bakteriol. **65**:403, 1912.
47. Winternitz, M. C., and Hirschfelder, A. D.: J. Exper. Med. **17**:657, 1913.
48. Lewis, P. A.: J. Exper. Med. **23**:669, 1916.
49. McClellan, R. H., and Goodpasture, E. W.: J. M. Research **44**:201, 1923.
50. Siengalewicz, S. S.: J. Pharmacol. & Exper. Therap. **24**:289, 1925.

nervous tissue, such as poisoning with carbon monoxide or with arsphenamine, was followed by marked staining of the damaged areas by trypan blue. Ramsdell [51] injected trypan blue into the veins of rabbits and guinea-pigs previously treated with foreign serum, and found that injection of the same serum into the skin of the ear immediately caused local infiltration of the dye into the adjacent tissue. She regarded the infiltration by the dye as an indicator of edematous changes resulting from toxic injury to the capillary endothelium.

Okuneff [52] found that a thermal irritant favored the passage of vital stains from the blood stream into the area heated. Kusnetzowsky [53] also observed that the local application to the skin of an irritant such as heat or mustard oil caused an accumulation of trypan blue in the inflamed area when the dye had previously been injected into the blood stream.

I [19] found that trypan blue introduced into the circulating blood rapidly entered the site of inflammation, staining the tissue deeply, and that it did not readily drain away through the tributary lymphatic vessels (fig. 1). I further observed that the longer the interval of time between the injection of the irritant and that of the dye, the more complete was its retention, less of the dye diffusing to the regional lymph node. Thus there was not only a rapid accumulation, but also a fixation of dye from the blood stream in the inflamed area.

It is well known that the retrosternal lymph nodes of the anterior mediastinum drain the peritoneal cavity. When trypan blue was injected into a normal peritoneal cavity the retrosternal lymph nodes were within a short time deeply stained by the dye, but when the dye was injected into a peritoneal cavity in which an inflammatory reaction had been set up by a sterile irritant, such as aleuronat, the dye was wholly or partly prevented from reaching the retrosternal lymphatic nodes. From these studies the following principle has consequently been established: A vital dye, trypan blue, injected into the circulating blood stream rapidly accumulates in an inflamed area and is fixed there, so that it fails to appear in the tributary lymphatics draining the area.

The accumulation in inflamed tissue of the dye that has been introduced into the blood stream is doubtless the result of the increased permeability of the capillaries which is part of the inflammatory reaction.[54] We were able to secure quantitative data by studying directly the change in the concentration of the dye in the blood stream both in the inflamed, and in the normal, mesentery of the frog. After intraventricular injection of the dye the change in concentration of trypan blue within the

51. Ramsdell, S. G.: J. Immunol. **15**:305, 1928.
52. Okuneff, N.: Arch. f. d. ges. Physiol. **204**:261, 1924.
53. Kusnetzowsky, N.: Ztschr. f. d. ges. exper. Med. **44**:646, 1925.
54. Menkin, V., and Menkin, M. F.: J. Exper. Med. **51**:285, 1930.

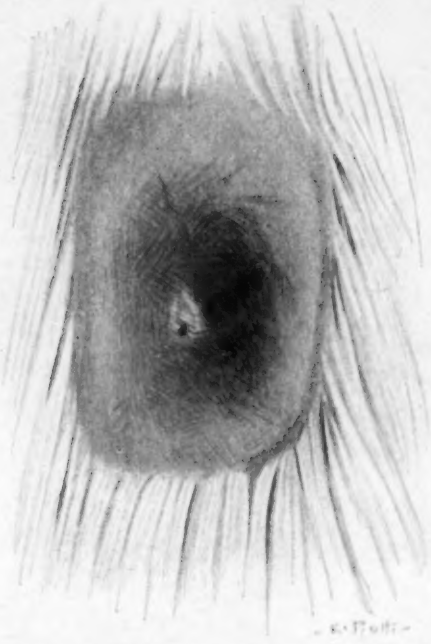

Fig. 1.—The accumulation of trypan blue at a site of inflammation when the dye was injected into the circulating blood stream. The area of inflammation, which was of four hours' duration, was induced in the skin of the abdomen of a rabbit by injection of concentrated broth. The dye was injected intravenously fifteen minutes after the injection of the irritant.

Figure 2

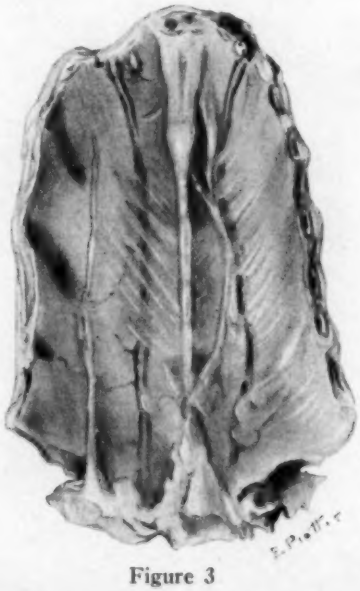

Figure 3

Fig. 2.—Fixation of injected iron by an inflammatory reaction. Ferric chloride was injected into the normal peritoneal cavity of a rabbit. One hour later the rabbit was killed. The substernal region revealed the presence of iron in the retrosternal lymphatics that drain the peritoneal cavity (prussian blue reaction).

Fig. 3.—Ferric chloride was injected into a peritoneal cavity that had been inflamed for twenty-two hours and forty minutes. An hour later the rabbit was killed. The substernal region failed to reveal the presence of iron to any appreciable extent in the lymphatics when the qualitative test was applied to the tissues.

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capillaries could be estimated by a colorimetric method. When the logarithms of the concentrations of the dye within the capillary were plotted against time, a straight line was obtained, denoting an exponential type of relationship. The equations derived by the method of least squares for both the normal and the inflamed areas were of the type:

$$y = be^{-ax}$$

The actual equations found were:

$y = 7.63e^{-0.24x}$ ..... (1) in the normal peritoneal cavity

$y = 6.9e^{-0.42x}$ ..... (2) in the inflamed peritoneal cavity

In these equations y represents the concentration of dye in the capillaries as estimated by comparison with the nearest standard on the colorimetric scale, and x stands for the time in minutes after the intraventricular injection of the dye, and a represents the slope of the curve. In the equation for the control experiments, a equaled 0.24, while in the equation for the concentration of dye in the capillaries of the inflamed peritoneal cavity, a equaled 0.42. Since a is an index of the slope and consequently of the rate of the change of concentration of the dye, it is clear that the rate of the fall of the concentration of trypan blue was almost twice as rapid in the capillaries of the inflamed area as in the capillaries of the normal mesentery. The dye obviously diffused outward into the extracapillary spaces, as could be seen by direct observation and of course by the fact that the cells of the extracapillary spaces were stained. This does not necessarily mean that the fall of the concentration of dye within the capillary is an exact measure of the amount of dye that passes through its walls, for it is conceivable that there may be other factors involved. However, in view of previous studies [19] showing that trypan blue injected intravenously rapidly passes into an inflamed area, it is believed that the increased rate of the fall of concentration is a measure of the increased passage of the dye through the wall of the capillary.

Landis showed that capillaries injured by alcohol and mercuric chloride are permeable to the plasma colloids and approximately seven times more permeable to fluids than the normal capillaries. It seems probable that similarly the inflammatory irritant may have a direct toxic effect on the walls of the capillaries and, by increasing their permeability, may cause a fall in the osmotic pressure of the plasma colloids. This direct injury would result in an increased rate of filtration of the dye into the inflamed area and would account for the increased rate of the fall in the concentration of the dye within the capillaries. Landis,[55] however, recently demonstrated that when a wheal is formed on the skin by freezing, or when a blister is caused by the application of a cantharidal plaster, the average capillary pressure at the point of injury rises appre-

55. Landis, E. M.: Heart **15**:209, 1930.

ciably. This factor in addition to increased permeability of capillaries also increases the filtration of substances into the extracapillary spaces.

In order to determine whether the accumulation of trypan blue from the circulating blood stream into an inflamed area is due to direct damage to the wall of the capillaries, by the contact of the irritant, the following experiment, suggested by Prof. S. B. Wolbach, was performed. A small area at the center of the cornea of an anesthetized rabbit was burned by the application of a red hot platinum wire. On the following day only the small central area of the injury in the cornea appeared cloudy. At the periphery, however, the tissues overlying the sclera were definitely congested and thickened. Ten cubic centimeters of 1 per cent trypan blue was injected intravenously. Within a very short time the tissues at the periphery of the cornea were deeply stained by the dye. The corresponding normal area in the other eye showed either no presence of the dye or only a minute trace of it. This experiment indicated that the capillaries at the site of inflamed tissue are more permeable to trypan blue than those in normal areas.

Recently Underhill and his collaborators [56] showed that whereas trypan blue injected intravenously rapidly permeates into the edematous part of an area of burned skin, the reabsorption of the dye from this area is a very slow process. On the basis of their observations these investigators concluded that increased permeability of the capillaries in one direction may exist simultaneously with decreased permeability in the opposite direction. The experimental evidence that I [57] presented, although not disposing of their contention, renders such an inference perhaps unnecessary. In the studies of lymphatics draining an inflamed area it was readily demonstrated that the failure of the dye to be reabsorbed from such an area was due to its fixation in situ by the presence of a fine network of fibrin and of thrombosed lymphatics.

Further studies were undertaken to determine whether a metal would, like the dye, be fixed in situ by the inflammatory reaction.[58] Iron was selected because of the ease of detecting this metal in tissues qualitatively by the prussian blue reaction. When colloidal iron or ferric chloride was injected into the normal peritoneal cavities of rabbits, it rapidly accumulated in the retrosternal lymph nodes, as was shown by the prussian blue reaction, but it failed to reach these lymph nodes when it was injected into peritoneal cavities in which inflammation had been caused previously either by aleuronat or by *Staph. aureus* (figs. 2 and 3). Quantitative studies by the method of Kennedy [59] of the iron con-

56. Underhill, F. P.; Kapsinow, R., and Fisk, M. E.: Am. J. Physiol. **95**:315, 1930.

57. Menkin (footnotes 54 and 19).

58. Menkin, V.: J. Exper. Med. **51**:879, 1930.

59. Kennedy, R. P.: J. Biol. Chem. **74**:385, 1927.

tent of these lymph nodes in animals into which ferric chloride had been injected intraperitoneally revealed 56.7 per cent more metal in the nodes of the animals with normal peritoneal cavities than in those with inflamed peritoneal cavities. Experiments were then performed to demonstrate the accumulation of the metal in inflamed areas when ferric chloride was injected into the circulating blood stream. Acute inflammatory reactions of from six to seven hours' duration were obtained by the injection of *Staph. aureus* into the skin of the abdomens of rabbits. Such acute inflamed dermal areas did not themselves give the prussian blue reaction, but when ferric chloride was injected intravenously the metal became demonstrable in these areas by qualitative tests. Quantitative determinations showed in inflamed areas of the skin of normal animals, as an average figure, 9.7 mg. of iron per hundred grams of dry tissue, as compared with 16.2 mg. in animals into which ferric chloride had been injected intravenously, or an increase of 67 per cent in iron content after the introduction of the ferric salt. The average iron content of normal areas of the skin in the animals receiving the injections was 10.4 mg., as compared with 8.4 mg. in the animals not given injections, showing an increase of 23.8 per cent as a result of the injection of the metal salt. Thus about three times more metal accumulated in the inflamed, than in the normal, areas of the skin. These observations show that iron accumulates, as does trypan blue, in an inflamed area when injected intravenously and is fixed there by the inflammatory reaction.

These studies may have a clinical application. It is conceivable that by the accumulation of dye, iron or other material in an inflamed area, the character or the course of development of the inflammatory reaction may be altered. I [60] demonstrated by qualitative and subsequently by quantitative methods that daily intravenous injections of ferric chloride in tuberculous rabbits caused an accumulation of iron in the caseous areas of the tuberculous foci.[60] Whereas in inflamed areas previously studied the iron salt had been found to accumulate at the periphery and not in the central portion (where the circulation is relatively inactive), these tuberculous foci, on the contrary, revealed the metal in the caseous centers. It is to be noted in this connection that various dyes that cannot penetrate living cells are able to stain dead or dying cells.[61] Further experiments are being conducted to determine the effect of the accumulation of iron in tuberculous areas on the course of development of the disease.

As mentioned, Opie [35] showed that foreign protein injected into the skin of an actively immunized animal is fixed at the site of injection,

60. Menkin, V.: Proc. Soc. Exper. Biol. & Med. **27**:1020, 1930. Menkin, V., and Menkin, M. F.: J. Exper. Med. **53**:919, 1931.

61. Stechelmacher, S.: Beitr. z. path. Anat. u. z. allg. Path. **57**:314, 1914.

where the contact of antigen and antibody causes an acute inflammatory reaction (Arthus' phenomenon). In view of this work on immunized animals and of the results obtained with trypan blue and iron, I [29] undertook experiments to determine whether a readily identified foreign protein, such as horse serum, injected into an area of inflammation caused by either bacteria or a sterile irritant would also be retained in situ by the inflammatory reaction.

Horse serum was injected into the peritoneal cavities of normal rabbits and of rabbits that had previously been given an intraperitoneal injection of an inflammatory irritant. The presence of the foreign protein was tested by the precipitin reaction on blood samples removed at varying intervals from the heart. It was found that horse serum injected into an inflamed peritoneal cavity penetrated into the blood stream less rapidly than when introduced into the normal cavity. Furthermore it was noted that when the foreign protein was injected into a cutaneous inflammatory area it was held in situ for a longer period than when injected into an inflamed peritoneal cavity.

These experiments showed that, like trypan blue and iron, complex foreign proteins, such as are found in horse serum, when injected into an inflamed area (caused in Opie's experiments by the contact of antigen and antibody and in the present experiments by a sterile irritant) are held fixed by the inflammatory reaction.

With the demonstration that the penetration of horse serum into the blood stream is delayed at the site of inflammation, the attempt was made to determine whether, as with trypan blue and ferric chloride previously studied, horse serum injected into the circulating blood stream would accumulate in inflamed areas to a greater extent than in normal tissue.

Rabbits were used. An area of cutaneous inflammation was induced by the injection of about 0.2 cc. of a saline suspension of *Staph. aureus* into the skin of the abdomen. About three hours later 10 cc. of horse serum was injected intravenously. The animal was killed when the inflammation was of from five to six hours' duration. Saline extracts obtained from the inflamed area and from an area of normal skin were tested for the presence of horse serum. It was consistently found that a greater concentration of the foreign protein could be recovered from the site of inflammation than from the corresponding area of normal skin. These results were therefore similar to those obtained with trypan blue and with ferric chloride. The accumulation of foreign protein in inflamed tissue is doubtless in part the result of the increased passage of fluid from the circulating blood stream, but the observations recorded indicate that its escape from the site of inflammation is retarded.

These observations offered a means of explaining an interesting observation made some years ago by Auer.[62] This investigator showed that when the ear of a rabbit immunized with horse serum was painted with xylene, following reinjection of the homologous antigen into the peritoneal cavity, an intense inflammatory reaction, followed by necrosis, took place in the affected ear. No such severe reaction was seen when xylene had been applied to the ear of a normal rabbit after a single injection of horse serum. In view of the observations on the accumulation of foreign protein in inflamed areas it is highly probable that the reaction in Auer's experiment was simply the result of an accumulation in the inflamed ear of the sensitized animal of antigen and antibody from the circulating blood stream. The contact of antigen and antibody in the tissues evidently caused an acute inflammatory reaction, thus intensifying the mild reaction produced by xylene alone. This explanation is verified by the demonstration in several experiments that horse serum from the circulating blood accumulates in greater concentration in the inflamed, than in the normal, ear of a sensitized animal.

It is also possible that the phenomenon of focal reaction in tuberculosis may be explained by these observations. When tuberculin is introduced into the blood stream of an animal with a tuberculous lesion, an intense inflammatory reaction may develop in the lesion. The mechanism of this focal reaction in tuberculosis is not understood. It is conceivable in view of the aforementioned experiments with horse serum that in a like manner tuberculoprotein from the blood stream may accumulate in the inflamed tuberculous lesion and by its presence there induce a local inflammatory reaction.

Shwartzman [63] described an interesting reaction that occurred when a filtrate of *B. typhosus* was injected into the skin of a rabbit. When twenty-four hours later the same filtrate or a filtrate of a nonrelated organism, such as the meningococcus, was introduced into the blood stream, hemorrhagic necrosis often took place at the site of the cutaneous injection. The observations on the accumulation and fixation of foreign proteins at the site of inflammation which have been described suggest that the intense cutaneous reaction following the intravenous injection of a bacterial filtrate may be the result of an accumulation of this substance in an area of the skin already inflamed, with resulting accentuation of the original lesion. The reaction does not have to take place with all bacterial filtrates or with all inflammatory irritants. The degree of permeability of the capillaries and the optimum of the synergistic action of two irritating substances on one another may modify the final reaction. On conceiving the phenomenon from this angle, it is

62. Auer, J.: J. Exper. Med. **32**:427, 1920.
63. Shwartzman, G.: J. Exper. Med. **48**:247, 1928.

suggested that it may be purely a nonspecific reaction resulting from the accumulation of an irritating substance in an inflamed area. Frisch [63a] recently showed that this reaction took place in the prepared area of the skin if the bacterial filtrate was injected intraperitoneally. When, however, the filtrate had been previously injected several times into the peritoneal cavity, the skin reaction failed to occur when the filtrate was injected intraperitoneally, but not when injected intravenously. The failure of the reaction may perhaps be explained in terms of fixation of the bacterial filtrate in a peritoneal cavity inflamed by preliminary injections of the filtrate.

Further studies were undertaken to determine the behavior of particulate matter and of bacteria at the site of inflammation when injected intravenously or directly into an inflamed area.[15] It was believed that by such studies additional valuable information might be obtained concerning the increased permeability of the capillaries in inflammation and the consequent localization of bacteria from the circulating blood stream when reaching a point of injury. Previous investigators had studied the dissemination of bacteria from the site of inflammation by recovering the organisms in the blood stream. The local state had not been studied. In experiments that I performed it was shown that the failure of bacterial dissemination from the site of inflammation was due to fixation of the micro-organisms in situ. It was found that diluted india ink or particles of graphite injected into a normal peritoneal cavity readily drained into the retrosternal lymph nodes. When, however, these particles were injected into an inflamed peritoneal cavity, they failed to disseminate to any extent to the tributary lymphatic nodes.

The attempt was next made to determine whether india ink injected into the circulating blood would rapidly enter the inflamed area. No satisfactory evidence to that effect could be obtained. It is well known in this connection that india ink adheres to the endothelial lining of capillaries. Kusnetzowsky,[64] however, reported that as the vessels become more permeable in areas of inflammation particles of india ink make their way through the endothelial wall into the extracapillary spaces, where they are taken up by the polyblasts. Graphite ink (Hydrokollag "300") has the advantage over india ink of not adhering to the vascular wall.[65] For this reason experiments were performed to determine whether such particles accumulate in an inflamed area when injected into the circulating blood stream. Rabbits were used. An inflammatory reaction was induced in the skin of the abdomen by any one of a variety of sterile or bacterial irritants. Several hours later 10 cc. of a

63a. Frisch, J. A.: Arch. Int. Med. **46**:361, 1930.

64. Kusnetzowsky, N.: Beitr. z. path. Anat. u. z. allg. Path. **83**:649, 1929.

65. Drinker, C. K., and Churchill, E. D.: Proc. Roy. Soc., London s. B. **101**: 462, 1927

diluted suspension of particles of graphite was injected into the aural vein. After a variable interval of time the area of inflammation revealed large deposits of particles of graphite. Histologic section of such an area showed many polymorphonuclear leukocytes loaded with graphite. The question arose as to whether particles of graphite injected into the circulating blood are brought to the site of inflammation entirely by leukocytes or whether the permeability of the capillary is also sufficiently increased to allow some of these relatively large particles to pass through the endothelial wall. To settle this point histologic examination was made of areas of skin in which the duration of inflammation was only of about six hours. Careful studies under oil immersion magnification revealed many capillaries with unattached particles of graphite within their lumina. Some of these particles were in the process of passing into the extracapillary spaces. In the tissue spaces many of these isolated granules were found surrounding the capillaries. Only occasionally in such regions could the particles be seen within polymorphonuclear leukocytes. It is evident, therefore, that when the inflammatory reaction is of short duration the passage of nonphagocytosed particles of graphite through the walls of capillaries can be readily demonstrated. Since no such evidence was obtained in sections of normal areas, the observations indicate that with inflammation the walls of the capillaries become permeable to particulate matter.

Experiments were devised to determine whether bacteria would be fixed like particles of carbon by the inflammatory reaction. The organism studied was *B. prodigiosus*. In a few experiments *B. pyocyaneus* was used. The organisms were injected into both the normal and the inflamed peritoneal cavities of rabbits. After a variable interval of time the retrosternal lymph nodes were removed, and the number of bacilli was determined by a culture method. It was found that whereas a large number of bacilli were recovered from the nodes in the normal animal, a relatively insignificant number were obtained from those of rabbits having inflamed peritoneal cavities.

Experiments were set up to determine whether or not this failure of bacterial dissemination to the tributary lymph nodes was actually due to retention of the micro-organisms at the site of inflammation. In all experiments a larger number of micro-organisms were recovered from the site of inflammation than from a corresponding normal area. It is to be noted that precisely similar results had been obtained with a foreign protein.[29] Rich and McCordock [27] questioned the view that acute inflammation per se can prevent the spread of bacteria. These observations represent direct evidence that bacteria are retained in situ by the inflammatory reaction, and consequently fail to disseminate to the tributary lymphatic nodes as readily as under ordinary circumstances.

It is known that injury to tissue may determine the localization in the damaged tissue of bacteria or of ultrafiltrable organisms present in the circulating blood. Calmette and Guérin [66] found that after the back of a rabbit had been shaved, intravenous injection of the virus of vaccinia resulted in a localization of the virus in the epilated area. Kettle [67] showed that tubercle bacilli injected intravenously into mice or rabbits tended to settle in localized subcutaneous lesions caused by various agents that produce increased vascularity and necrosis of tissue. Chesney, Turner and Halley [68] found that when rabbits with wounds in their backs were inoculated either intratesticularly or intravenously with *Spirochaeta pallida,* syphilitic lesions invariably developed in the wounds. Findlay [69] believed that histamine-like substances, liberated at the point of injury, were the specific agents, which by their action on the walls of the capillaries caused organisms present in the blood stream to localize at the site of injury. Sager and Nickel [70] recently demonstrated that subcutaneous injection of silver nitrate into rabbits caused sterile abscesses. After intravenous injection of green streptococci, these organisms were recovered from the abscesses in some of the rabbits. They concluded that bacteria may become localized in places of lowered resistance. Quednau [71] pointed out that bacteria, especially pneumococci, in the blood stream tended to localize readily in areas of cerebral softening.

I undertook experiments to determine whether bacteria which, when injected directly into an inflamed area, are fixed in situ would accumulate rapidly at the site of inflammation when injected into the blood stream.[15] Rabbits were used. An area of inflammation was induced by injecting 0.5 cc. of concentrated broth in the skin of the abdomen. After a short time a suspension of *B. prodigiosus* or of *B. pyocyaneus* was injected intravenously. When a variable interval of time had elapsed, the animal was killed and the number of micro-organisms localized in both the inflamed and a normal area of the skin was determined by culture of the respective tissues. The number of intravenously injected bacteria accumulating at the site of inflammation was found to be distinctly greater than that in an area of normal skin. These observations are in agreement with the results described in which it was demonstrated that

66. Calmette, A., and Guérin, C.: Ann. Inst. Pasteur **15**:161, 1901.

67. Kettle, E. H.: Brit. J. Exper. Path. **5**:158, 1924.

68. Chesney, A. M.; Turner, T. B., and Halley, C. R. L.: Bull. Johns Hopkins Hosp. **42**:319, 1928.

69. Findlay, G. M.: J. Path. & Bact. **31**:633, 1928.

70. Sager, W. W., and Nickel, A. C.: Arch. Surg. **19**:1086, 1929.

71. Quednau, F.: Beitr. z. path. Anat. u. z. allg. Path. **83**:471, 1929.

the permeability of the capillaries in inflamed areas is sufficiently increased to allow particles of carbon to pass through the endothelial wall.

The frequent localization of bacteria from the blood stream in a *"locus minoris resistentiae"* occurring after preliminary trauma or other injury of tissue is well known to pathologists and clinicians. My observations [15] on the accumulation of bacteria in an inflamed area from the circulating blood stream may explain the mechanism of this phenomenon in terms of increased permeability of the capillaries, with resulting accumulation and fixation of bacteria at the point of injury.

THE MECHANISM OF FIXATION BY THE INFLAMMATORY REACTION

With the demonstration that the accumulation of foreign substances from the circulating blood in an inflamed area is associated with increased permeability of the capillaries, studies were extended in an attempt to determine the mechanism of the fixation by the inflammatory reaction.[4a]

The leukocytes are probably not a very significant factor in the mechanism of fixation, for two reasons. In the first place, no definite histologic evidence could be obtained of phagocytosed particles in the leukocytes of the inflamed area at a time when fixation of foreign substances was demonstrable by examination of the tributary lymphatics. In the second place, fixation of trypan blue at the site of inflammation occurred as early as thirty minutes after the injection of the inflammatory irritant.[19] The occurrence of fixation at this early stage of the inflammatory reaction when there were as yet relatively few leukocytes present seems to point toward some other agent responsible for fixation.

A factor that might explain fixation is mechanical obstruction. It is conceivable that a network of fibrin and thrombosed lymphatics at the site of inflammation might arrest the passage of particulate material injected into the inflamed area. The dissemination of fluids would probably also be retarded by mechanical obstruction of this kind, though probably not as effectively as that of solid particles, which would be more readily caught in a fibrinous network. In this connection it is interesting to note that some years ago Opie [72] showed that when cantharidin was administered intramuscularly, the flow of lymph in the thoracic duct was at first diminished, but later might be increased. The decrease in the flow of lymph was accompanied by acute edema of the liver and gallbladder. This edema was due to a plugging of the afferent

72. Opie, E. L.: J. Exper. Med. **16**:831, 1912.

lymphatic vessels and the sinuses of lymph nodes that drain these organs by fibrin. The observations of Adami mentioned at the beginning of this review are also significant in this connection.

In the endeavor to throw some light on the mechanism involved in fixation, a series of experiments was undertaken to determine whether the inflammatory exudate in itself possesses some property that might facilitate the fixing of foreign substances in the inflamed area.

It was found [73] that when ferric chloride was added directly to the inflammatory exudate heavy precipitation occurred. The compound formed was presumably a ferric proteinate.[74] When horse serum was added to the exudate and incubated at 37 C. for a short time, coagulation took place. However, when trypan blue was added to an inflammatory exudate, no effect was noticed. Yet this vital dye was shown to be definitely held in situ by the inflammatory reaction. The reaction of fixation must be due primarily to some mechanism other than precipitation or coagulation of foreign substances by the inflammatory exudate. As it was shown, however, that iron compounds are apparently more effectively held by the inflammatory reaction than trypan blue, it is possible that precipitation or coagulation of foreign substances acts as a secondary factor in the mechanism of fixation by preventing rapid dissemination from the site of inflammation.

Sections were made of the inflamed tissues of rabbits in experiments in which either trypan blue or ferric chloride had been shown to be fixed in situ by the inflammatory process. In these sections there is shown, as a rule, a central area of dense leukocytic infiltration. The intensity of the inflammatory reaction adjacent to veins and arteries is noteworthy. It is to be recalled [19] that when the dye was injected intravenously, it would not always penetrate into the central zone of the inflamed area. This was evidently due to thrombosis of the small blood vessels, for sections of such areas reveal some thrombosed vessels with acute inflammatory changes in the surrounding tissue.

Histologically there is little evidence of phagocytosed particles of trypan blue or of iron within the leukocytes at a time when retention of these substances at the site of inflammation is clearly demonstrable.

A mesh of fibrin is usually found at the periphery of the zone of dense infiltration (fig. 4). In the same region careful study reveals many lymphatic vessels that are thrombosed; one of these is illustrated in figure 5. The thrombus is characterized by numerous leukocytes within a delicate fibrinous reticulum. The fact that there are many occluded lymphatics and a dense network of fibrinous strands within tissues that are distended with edema at the site of inflammation sup-

73. Menkin (footnote 72, first reference).

74. Smythe, C. V., and Schmidt, C. L. A.: J. Biol. Chem. **88**:241, 1930.

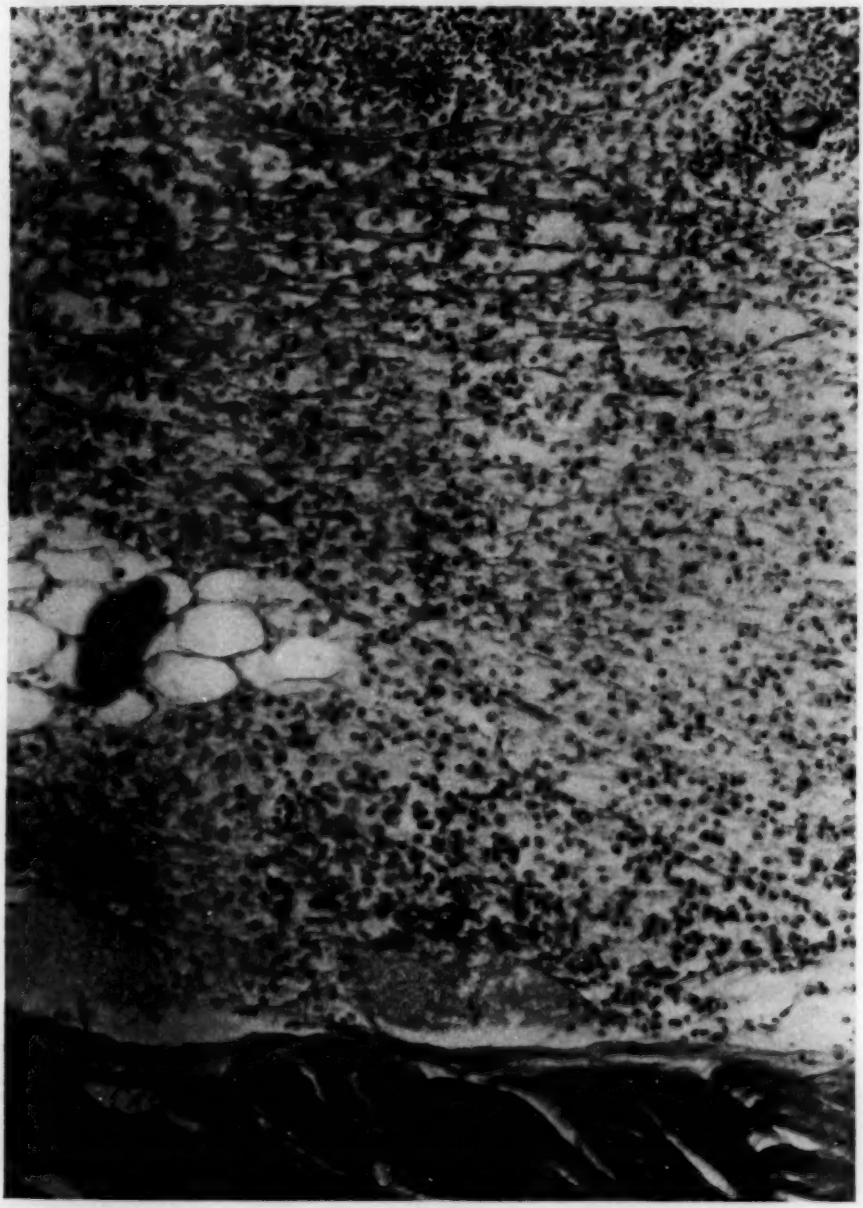

Fig. 4.—The mesh of fibrin at the periphery of a zone of infiltration.

ports the view that foreign substances, especially solid particles, such as precipitated iron salts, can disseminate only with difficulty from the inflamed area through the regional lymphatic vessels.

If, as described, the thrombosed lymphatics and the network of fibrin in the acutely inflamed area are instrumental in mechanically preventing the free passage of substances from the site of inflammation, it

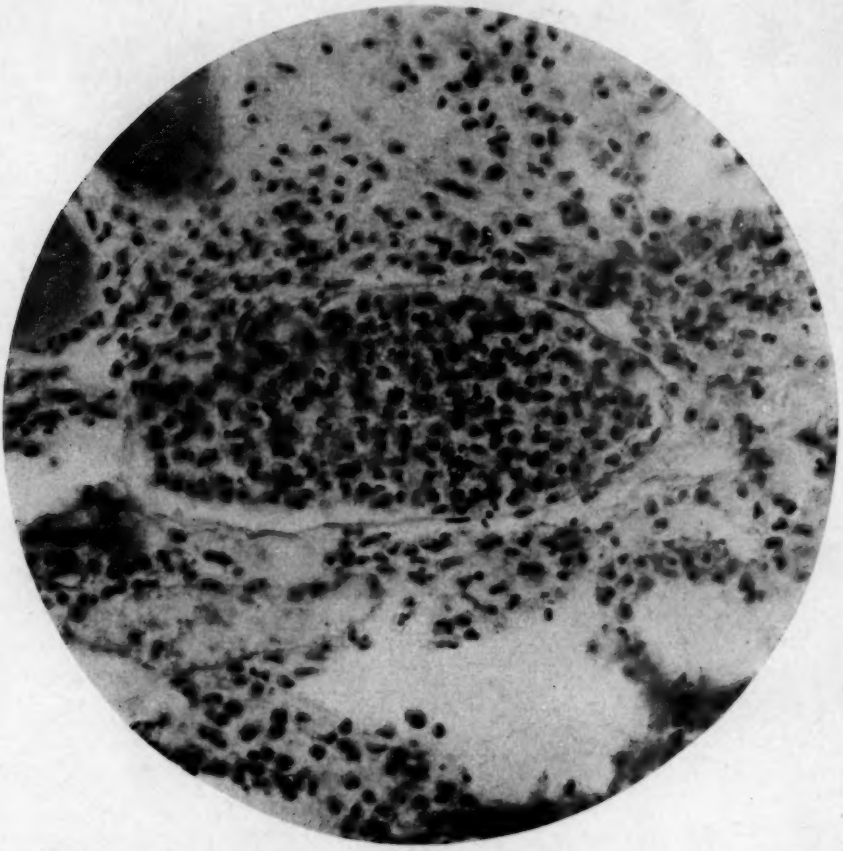

Fig. 5.—A thrombosed lymphatic vessel in a zone of infiltration (from the Journal of Experimental Medicine).

follows that similar substances injected at the periphery of the inflamed area should be prevented by the same obstruction from entering it. To test this hypothesis, the following experiments were conducted at the suggestion of Professor Opie.

An inflammatory reaction was induced in the skin of the abdomen of a rabbit by the use of a bacterial irritant (*Staph. aureus*) or by the injection of concentrated broth. After a varying interval of time trypan

Fig. 6.—The failure of trypan blue to penetrate a site of inflammation when injected at its periphery: 0.1 cc. of trypan blue was injected into each of several areas surrounding an inflamed area. About one and a half hours later the dye had diffused into the surrounding normal tissues, but had failed to penetrate the site of inflammation.

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blue was injected at the periphery of the inflamed area in from four to six places. In this way the inflamed area became circumscribed by a blue band. In an area of normal skin similar injections of dye were made to serve as control. Several hours later the inflamed area within the original circumscribed blue band showed no trace of dye, whereas the area of normal skin was diffusely blue (fig. 6). The dye had evidently failed to penetrate into the site of inflammation, when injected at its periphery, owing to the presence of thrombosed lymphatics and of a fine network of fibrin in the tissue spaces. Experiments of the same type repeated on frogs yielded similar results.

Direct observation with a binocular dissecting microscope demonstrated [75] that in frogs the failure of the dye to penetrate into an inflamed area was due to the mechanical obstruction of the lymphatic vessels by blood clots; histologic sections revealed a network of fibrin in the tissue spaces of the inflamed areas.

Similar results were obtained with bacteria.[15] When *B. prodigiosus* was injected at the periphery of an inflamed area the micro-organisms failed to penetrate to any appreciable extent into the site of inflammation; but they disseminated rapidly into normal tissue.

Fixation of foreign substances by the inflammatory reaction is therefore primarily due to mechanical obstruction caused by a fine network of fibrin and by thrombosed lymphatics at the site of inflammation. Further experiments are being conducted to determine the relation between exudation from blood vessels and change in the flow of lymph from the site of inflammation.

The experiments described present proof that various foreign substances, including a dye, a metallic salt, a foreign protein, particulate matter and bacteria, injected into the site of inflammation are fixed in situ and fail to drain readily into the tributary lymphatic vessels. These same substances injected intravenously accumulate rapidly in inflamed areas. This accumulation is partly associated with increased permeability of the capillaries, but is also the result of the inability of these substances to escape from the site of inflammation, owing to the presence of a fine network of fibrin and of thrombosed lymphatics. The mechanism of fixation takes place extremely early in the inflammatory process. In previous experiments [19] fixation of a dye at the site of inflammation could be demonstrated by study of the regional lymphatics as early as thirty minutes after the injection of the inflammatory irritant. This would indicate that the earliest change in inflammation is an increase in the permeability of the capillaries which permits the passage of fibrinogen from the plasma into the tissue spaces. The rapid formation of a network of fibrin and of thrombi in lymphatics at the site of inflam-

75. Menkin (footnote 4a., second reference).

mation circumscribes the irritating substance and thus prevents its passage into the blood stream. This allows a definite interval of time for the leukocytes to assemble for phagocytosis. The initial fixation of bacteria or of other injurious substances at the site of inflammation thus becomes a protective mechanism and plays a definite rôle in immunity.

Dr. S. B. Wolbach supplied the photomicrographs and Miss E. Piotti the colored plates.

Notes and News

University News, Appointments, Promotions, Resignations, Deaths, etc.—R. E. Miller has been appointed assistant professor of pathology in the Dartmouth Medical School, Hanover, N. H.

E. B. McKinley, professor of bacteriology in Columbia University and director of the Porto Rico School of Tropical Medicine, has been appointed dean of the school of medicine of the George Washington University, Washington, D. C.

James R. Cash, formerly professor of pathology in Peiping Union Medical College, now occupies the Walter Reed professorship of pathology in the University of Virginia, vacated by the death of Harry T. Marshall.

D. H. Bergey, formerly professor of hygiene and bacteriology in the University of Pennsylvania, has been appointed director of research and biology by the National Drug Company.

J. C. Geiger, professor of epidemiology in the University of California, has been appointed health officer of the city and county of San Francisco.

LaVerne A. Barnes, senior instructor in bacteriology in the school of medicine of Western Reserve University, has resigned to become immunologist to the state department of health of Massachusetts.

Newell R. Ziegler has been appointed associate professor of bacteriology in the University of Missouri.

J. F. Rinehart has been appointed assistant professor of pathology in the University of California.

H. E. Teasley has been appointed instructor in bacteriology in the University of Colorado.

William Freeman has been appointed pathologist at the Worcester State Hospital, Worcester, Mass.

It is reported that Carl V. Weller has been appointed professor of pathology and director of the pathological laboratory at the University of Michigan to succeed the late Aldred S. Warthin.

The Baly Medal of the Royal College of Physicians of London, conferred alternate years on the person deemed to have distinguished himself most in the science of physiology during the two immediately preceding years, has been awarded to W. B. Cannon, professor of physiology in the Harvard Medical School.

The three hundred thousandth microscope made by the firm of E. Leitz has been presented to Prof. Ludwig Aschoff, Freiburg, Germany.

A. F. DeGroat has been appointed assistant professor of pathology in the University of Arkansas.

Society News.—During 1932 the Pathological Society of Great Britain and Ireland will hold a meeting at Guy's Hospital in January and at Oxford in the summer.

The German Pathological Society will meet in Rostock during the week of May 14 to 21, 1932, under the presidency of G. Herxheimer. The subjects for special reports are: (1) The Pathologic Anatomy of the Spinal Column, referee, G. Schmorl, and (2) Glioma, referee, Folke Henschen. According to the last report, the society now has 406 members.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

THE BEHAVIOR OF LEAD IN THE ANIMAL ORGANISM. ROBERT A. KEHOE and FREDERICK THAMANN, Am. J. Hyg. **13**:478, 1931.

The experiments recorded in this article show that tetra-ethyl lead is absorbed through the skin. The initial distribution of the lead in the tissues in rapid tetra-ethyl lead absorption corresponds to that of an oil-soluble material, and indicates therefore that some portion of the tetra-ethyl lead is absorbed and circulates as such. However, tetra-ethyl lead is rapidly decomposed by the tissues, including the skin, so that only a small portion of the lead found later in the blood is in the form of tetra-ethyl lead. As a net result, after a period of from three to fourteen days, all of the lead in the animal tissues is distributed in a manner characteristic of water-soluble lead compounds. In small dosages, the factors of rapid decomposition and low concentration in the blood so interfere with the distribution of tetra-ethyl lead as such, as to prevent its primary absorption by the nervous system. Even when absorbed as the terta-ethyl compound, the excretion follows quantitatively that of water-soluble lead compounds. Tetra-ethyl lead poisoning is therefore not different from lead poisoning occasioned by other lead compounds. Evidence is adduced showing that the absorption of tetra-ethyl lead from gasoline in concentrations not in excess of 0.1 per cent is inappreciable.

AUTHORS' SUMMARY.

ABSORPTION OF CALCIUM FROM THE GALL BLADDER. EDMUND ANDREWS and LEO HRDINA, Am. J. M. Sc. **181**:478, 1931.

In obstruction of the cystic duct in dogs, the relatively high calcium content of the bile of the cystic duct is gradually lowered, and in the presence of infection it is rapidly lowered.

AUTHORS' SUMMARY.

THE RÔLE OF DIET IN PROTECTING AGAINST LIVER INJURY BY ARSPHENAMINE. E. B. CRAVEN, JR., Bull. Johns Hopkins Hosp. **48**:131, 1931.

A diet high in fat and one high in protein protect against injury to the liver by arsphenamine, the diet high in fat being the more effective. A diet high in carbohydrate and starvation favor the injurious action of arsphenamine.

PATHOGENESIS OF MULTIPLE SCLEROSIS. RICHARD M. BRICKNER, Bull. Neurol. Inst., New York **1**:105, 1931.

In a previous contribution (*Arch. Neurol. & Psychiat.* **23**:715, 1930) Brickner came to the conclusion that the serum of patients suffering from multiple sclerosis contains a substance that destroys the myelin; it causes myelinolysis. The hypothetic substance was supposed to be a lipase. Brickner studied fifty-one patients with multiple sclerosis and seventy-nine normal persons to test this hypothesis. From his laborious and highly technical study, Brickner concludes that the blood of a patient with multiple sclerosis contains an abnormal lipase, and that most likely one does not deal in this disease with a filtrable virus. The lipase is supposed to be the same agent that produces myelinolysis in the spinal cord in rats. Some of the facts established by the present studies are that in multiple sclerosis acid is produced in the blood serum, and that the production of acid can be inhibited by the addition of sodium oxalate. In lecithinated serum, acid is formed from preparations of the serums of both normal persons and patients with multiple sclerosis, but it is greater in the serum of the latter and may be markedly inhibited by oxalate.

GEORGE B. HASSIN.

THE INFLUENCE OF THE INTRAVENOUS INJECTION OF UREA ON THE EXCHANGE OF SUBSTANCES BETWEEN THE BLOOD AND THE TISSUES. HUGH DONOVAN and O. BRENNER, Brit. J. Exper. Path. **11**:419, 1930.

The results of the intravenous injection of 5 Gm. of urea in health and in various diseases were investigated. By the time the urea had mixed with the blood (three minutes), 80 per cent had left the blood stream. It is suggested that the urea leaves the blood by a process of simple diffusion, passing into the water of the cells, as well as into the free water, and that the process is complete in fifteen minutes, when the concentration of urea in the blood equals that in the tissues. A review of the literature indicates that urea is unique in this respect. With no other substance investigated is the distribution uniform over blood and tissues, and with most substances, the concentration in some tissues is greater than that in the blood, showing that the process by which the substance enters the tissues is not one of simple diffusion alone. It is suggested that the sudden introduction of urea into the body delays the formation of urea, and may even bring it to a standstill for the first two hours after the injection. The importance of this in connection with the production of uremia is mentioned. The osmotic attraction of the urea injected is greatly reduced by its rapidly leaving the blood. Salt also leaves the blood, so that the osmotic attraction of dissolved substances in the blood is not raised even three minutes after the injection. In spite of this the blood is more dilute at the end of three minutes. It is suggested that this may be due to a lag in the movement of water behind that of urea and salt. Evidence is discussed in favor of the view that, except in edematous patients, injection of urea causes some salt to pass out of the fluid of the body, to be stored in a "dry" form in the salt depots of the body, thus reducing the crystalloid concentration of the fluids of the body, which had been raised by the injection of urea; but that in most cases of edema, salt is liberated from the depots and enters the fluids of the body. It is pointed out that the capillaries are completely permeable to water and to most of the substances dissolved in the blood plasma, and are partly permeable to the plasma proteins. The factors in the retention of the blood inside the vessels are discussed. None of the causes ordinarily mentioned seem sufficient. It seems probable that the true cause must be sought in the tissues, rather than in the blood or in the blood vessels. It is suggested that perhaps the amount of fluid leaving the blood is limited by the capacity of the tissues for taking up water, and that this in its turn may be influenced by changes in the balance between acid and base, or by alterations in the relative proportions of various ions. A review of the literature shows that in experimental edema usually less fluid passes into the tissues, after intravenous injection, than in nonedematous animals, suggesting that an important factor in the production of edema is a deficient power of the tissues to hold and bind water, which therefore accumulates in the tissue spaces. Our own results suggest that in edema the tissues are also less able to bind salt, which also accumulates in the free fluids of the tissues. Any satisfactory theory of edema must account for this.

AUTHORS' SUMMARY.

THE SIGNIFICANCE OF THE LUNGS FOR THE TRANSPORTATION OF MATERIAL WITHIN THE BODY. P. SPANIER, Beitr. z. Klin. d. Tuberk. **76**:507, 1931.

The lungs retain a considerable number of leukocytes that have phagocytosed foreign particles. Such particulate matter is finally excreted again, but the roads of transport are not definitely known. Experiments are being made to use this leukocytic carrying mechanism in order to concentrate therapeutic agents in the lung tissue.

MAX PINNER.

THE ACTION OF THE BLOOD OF PREGNANT WOMEN ON FROG LARVAE. H. EUFINGER, H. WIESBADER and N. SMILOVITS, Klin. Wchnschr. **10**:348, 1931.

The blood of pregnant women fed to frog larvae influences their metamorphosis in the same way, if not as profoundly as does thyroxin. This effect is correlated with hyperfunction of the thyroid gland during pregnancy.

EDWIN F. HIRSCH.

FUNCTIONAL DISTURBANCES IN THE CENTRAL NERVOUS SYSTEM IN CEREBRAL ANEMIA. J. R. PETROFF, Ztschr. f. d. ges. exper. Med. **75**:1, 1931.

Experiments and observations are cited which show that marked disturbances in respiration are accompanied by definite changes in the vasomotor center. Stimulation of the respiratory center in repeated cases of cerebral anemia is accompanied by simultaneous stimulation of the vasomotor center. The vasomotor center is sensitive to changes in blood pressure in the brain. The author's experiments do not confirm the belief that carbon dioxide and substances such as lactic acid stimulate the vasomotor center and immediately release a pressor effect. The pressor action in asphyxia appears to result from stimulation of the respiratory center. Experiments are also described that show the difference between the action of the vasomotor center (vasoconstriction) and other central activity, such as the control of vascular tone.

PEARL ZEEK.

EXPERIMENTAL OBSTRUCTIVE JAUNDICE. B. VARELA FUENTES, J. DUOMARCO and A. MUNILLA, Ztschr. f. d. ges. exper. Med. **75**:577, 1931.

Changes in the glycogen metabolism of the liver following obstruction of the common bile duct were studied in dogs. Although the total amount of hepatic glycogen was found to be decreased, the liver was still capable of building and storing glycogen from excessive carbohydrate taken in food. The hyperglycemia following injections of epinephrine in these animals was similar to that in normal dogs, except that the height of the sugar content of the blood was reached after a somewhat longer interval. The curve of glycosuria following the injection of epinephrine was also essentially the same.

PEARL ZEEK.

THE IMPORTANCE OF SPLENOMEGALY IN ESSENTIAL THROMBOPENIA. R. BERGQVIST, Acta path. et microbiol. Scandinav. **8**:1, 1931.

Essential thrombopenia is ascribed by some investigators to disturbances in the marrow, and by others to excessive destruction of platelets in the spleen. The latter view seems to be supported by the good effects of removing the spleen in typical cases of the disease. Bergqvist describes the spleen removed in two typical instances: Grossly, the organ was moderately enlarged; microscopically, there was hyperplasia of germinal centers in follicles and pulp cells and the sinus endothelium, with indications of myeloid transformation (oxydase-positive granules). The view is advanced that the spleen somehow is directly concerned in essential thrombopenia, but its precise rôle cannot be defined.

XANTHOMATOSIS WITH CRANIAL DEFECTS (SCHÜLLER'S DISEASE). FREMANN-DAHL and FORSBERG, Norsk mag. f. lægevidensk. **92**:523, 1931.

In the form of xanthomatosis called Schüller's disease the xanthomatous infiltrations on the inner side of the cranium lead by pressure atrophy to the destruction of bone. In a girl, aged 14, with a history of trauma against the left temple at the age of 5, attacks of pain in the left temporal region set in three years later, which gained in frequency and intensity, and were followed by symptoms of slowly increasing intracranial pressure, together with diabetes insipidus, exophthalmos (left) and retardation of growth. Roentgen examinations showed the development of numerous sharp defects in the cranium, localized especially in the squamous portion of the temporal bone and also in the anterior part of the skull and left orbit, and revealed a typical maplike skull (Schüller). The diagnosis of xanthomatosis was supported by a negative Wassermann reaction, the presence of Bence-Jones protein in the urine, and by high blood cholesterol, pointing to a disturbance in the lipoid metabolism.

Pathologic Anatomy

MALIGNANT ENDOCARDITIS (PNEUMOCOCCAL), WITH EARLY CALCIFICATION AND CALCAREOUS RENAL EMBOLI. E. R. CULLINAN and W. S. BAXTER, Am. Heart J. **6**:420, 1931.

In a case of malignant endocarditis in a youth of 19 years, autopsy twenty days after the onset revealed calcification in the newly formed vegetations of the infected valve. The arterioles of the kidney contained calcified emboli. These had caused, not infarction by occlusion, but hemorrhage by trauma, in the adjacent renal substance.

ALFRED M. GLAZER.

SCLEROSIS OF SMALL MESENTERIC VESSELS WITH ULCERATION AND GANGRENE OF THE ENTERIC TRACT. PEARL ZEEK and JOHN J. PHAIR, Am. J. M. Sc. **181**: 548, 1931.

Three cases of extensive ulceration and gangrene of the gastro-enteric tract are described, in which these lesions are attributed to mesenteric arteriosclerosis.

AUTHORS' SUMMARY.

RENAL CALCIFICATION. S. PETRASSI, Arch. di path. e clin. med. **10**:104, 1930.

The various forms of renal calcification are classified by the author not only by topographic differences, but also by pathogenesis. Special attention is given to the "metastases of calcium" in the kidney. Calcifications in the kidney were found especially in chronic glomerulonephritis and primary sclerosis. The calcium can be found in the form of stones in cortical cysts, granules in hyalinized glomeruli, deposits in normal glomeruli and deposits in the arterial wall. The author believes that the majority of these calcium deposits are a sign of disturbed elimination of the calcium through the tubular epithelium.

E. HAAM.

EXPERIMENTAL PRODUCTION OF ENDOMETRIOSIS. C. R. FUMAGALLI, Riv. di pat. sper. **6**:154, 1931.

The three theories of the origin of endometriosis are discussed. The object in the experiments has been to test the theory of the endometrial origin. The endometrium of an amputated end of the uterus of the dog was transplanted in the ovaries and disseminated over the peritoneum. It was found that the endometrial cells can attach themselves easily and form growths of the general structure seen in human endometriomas.

E. HAAM.

PATHOLOGIC ANATOMY OF TUBERCULOSIS IN CHILDREN. H. KUDLICH, Beitr. z. Klin. d. Tuberk. **75**:575, 1930.

In Ghon's institute, in the years from 1926 to 1929, autopsies were performed on 136 children with tuberculosis. In 90 per cent of these cases, the primary infection occurred by way of the lungs. In 2.5 per cent there was a primary intestinal infection. In a girl, 6 years old, a partly calcified subpleural nodule was found, which had the appearance of a primary focus, but no lesion could be found in the regional lymph nodes. Microscopic examination showed that this nodule was not caused by tubercle bacilli, but by an unidentified parasite. Two cases with typical primary foci showed no lymphoglandular component ("rudimentary primary complex"). The localization of the primary foci was in general the same as described by Ghon. About one fourth of all primary foci showed excavation; this was noted in half of all infants. It is emphasized again that all primary pulmonary foci are exudative lesions, and that all evidence favors Ghon's opinion that the causative bacilli reach the alveoli by the respiratory tubules, and not by the blood or by the lymph stream. In 49 of the 136 children with tuberculous lesions, tuberculosis was not the cause of death. Chronic, isolated phthisis (the adult type)

was found in 4 children (ages 7½ months, 1½, 12 and 13 years). Seventy-four of the bodies revealed hematogenous propagation; 54 of these children had died of tuberculous meningitis. Six cases of so-called "endogenous lymphoglandular reinfection" are described in some detail.

MAX PINNER.

APICAL PLEURAL INDURATIONS. C. BOHNE, Beitr. z. Klin. d. Tuberk. **76**:164, 1930.

Apical pleural indurations can be differentiated macroscopically into three groups: (1) diffuse adhesive apical pleuritis, (2) localized adhesive pleural scar and (3) cartilage-like induration of the apical pleura. Histologic examinations showed that in the first variety small tuberculous foci are found in the thickened pleura. The subpleural tissue shows usually tuberculous processes, either scarred or active. This type was found in 10.1 per cent of the cases. The second type is usually seen in the area of the apical or subapical bronchiolus. The underlying pulmonary parenchyma usually shows anthracotic scars. These reveal microscopically either a typical Puhl focus or a caseous bronchitis. In the majority of these cases, however, the parenchyma shows only a hyaline scar in which no specific alterations are seen. This type of lesion was seen in 31.8 per cent of the cases. The third type is a hyalinized induration of the visceral pleura without involvement of the parietal pleura. The underlying pulmonary tissue is collapsed and anthracotic. Tuberculous foci are absent. The frequency of nontuberculous pleural indurations is emphasized.

MAX PINNER.

A RARE DIAPHRAGMATIC ANOMALY. W. PUTSCHAR, Centralbl. f. allg. Path. u. path. Anat. **50**:97, 1930.

A combination of diaphragmatic fenestration and hernia is reported in the body of a new-born girl. The right half of the diaphragm was normal; the left was partially absent in the anterior part and allowed herniation of the left lobe of the liver, stomach, tail of the pancreas, spleen and large and small bowel into the left pleural cavity, in part covered by thin diaphragm. Instead of the usual arrangement, in which all muscle strands in the diaphragm radiate to the central tendon, the only well developed muscle band in this body ran from the ribs to the vertebral column. Anterior to this band the left leaf of the diaphragm was thin and fenestrated, with muscle bundles occasionally in evidence; dorsal to this band the diaphragm was composed only of fused pleura and peritoneum. In addition to the aforementioned anomalies there were a mobile duodenum, a common mesentery for the small and large bowel and atypical peritoneal bands between the undersurface of the liver and the right kidney, and between the duodenum, cecum and appendix.

GEORGE RUKSTINAT.

FATTY CHANGES IN THE PULMONARY VEINS. R. PRÉVÔT, Centralbl. f. allg. Path. u. path. Anat. **50**:305, 1931.

Marked fatty changes in the pulmonary veins were found in eight and moderate fatty changes in thirty of sixty bodies, ranging in age from 4 months to 86 years. The changes were classified as occurring in elastic or nonelastic tissues, and those occurring in elastic tissues were predominant. A possible etiologic relationship was investigated in acute and chronic pulmonary, renal, cardiac and vascular disease. The only disease seeming to bear such a constant relationship was sclerosis of the pulmonary arteries.

GEORGE RUKSTINAT.

A PERICARDIAL DIVERTICULUM. H. P. YPSILANTI, Centralbl. f. allg. Path. u. path. Anat. **50**:417, 1931.

A pericardial diverticulum 9 by 3 by 1 cm., communicating with the pericardial sac through an opening 0.5 cm. in diameter, was found 2 cm. below the mouth of the inferior vena cava, in the body of a 34 year old man who had died of pul-

monary tuberculosis. An acute pericarditis was present in both the main portion of the sac and the diverticulum. There were no adhesions to indicate traction as a factor in the production of this condition.

GEORGE RUKSTINAT.

TWO GALLBLADDERS IN ONE PERSON. A. J. STRUKOW, Centralbl. f. allg. Path. u. path. Anat. **50**:420, 1931.

Two gallbladders, each 5 cm. long, were found attached to the liver of a 14 months old child who had died of scarlet fever. The cystic duct of the right bladder was 1 cm. long; that of the left, 1.5 cm. long. The openings of these into the common bile duct were 1 cm. apart. The cystic artery divided into a branch for each bladder at the level of its neck. No other abnormalities were found in the body.

GEORGE RUKSTINAT.

FOUR EXTRASELLAR HYPOPHYSEAL TUMORS. E. DEÁK, Centralbl. f. allg. Path. u. path. Anat. **51**:1, 1931.

Deák examined four hypophyseal tumors, which varied in size from that of a pigeon's egg to that of a hen's egg. These were highly cellular, the cells resembling the chief variety seen in the hypophysis. Graz, where the aforementioned material was obtained, is notorious for its endemic goiter. The author is of the opinion that a thyroid gland insufficiency might lead to hypophyseal hyperplasia. In only one of the bodies examined was a goiter present.

GEORGE RUKSTINAT.

RUPTURED ANEURYSM OF THE DUCTUS ARTERIOSUS. A. GUGGENHEIM, Frankfurt. Ztschr. f. Path. **40**:436, 1930.

A case of ruptured aneurysm is reported in a girl 20 months of age. The aneurysm had ruptured into the lower lobe of the left lung and into the left pleural cavity. In addition, the case revealed an empyema of the left knee joint. Histologically, the ductus arteriosus in the region of the rupture revealed a hemorrhage of the intima in several places. The intima was thickened, and the media was markedly fibrosed. The point of rupture could be demonstrated in an area 2 mm. distant from the aorta. The author states that, on account of the empyema of the knee joint, it is possible that this is a case of mycotic aneurysm, even though the inflammatory changes in the wall of the ductus arteriosus were very slight. Streptococci could be cultivated from the pus of the knee and from the spleen.

O. SAPHIR.

TWO CASES OF CYSTS OF THE PANCREAS. T. REEKE, Frankfurt. Ztschr. f. Path. **40**:444, 1930.

The first case reported was one of cystoma of the pancreas. It was found incidentally in a woman 76 years of age; it measured 1.5 by 2.4 cm. Microscopically, several small cysts were found, some of which had fused. The cysts were lined by one layer of flat epithelial cells. There was no evidence of malignancy. The tumor was well encapsulated. The second case was that of a woman of 54 with a cyst of the pancreas, the size of a child's head. The content of the cyst consisted of partly liquid, partly clotted, blood, with much necrotic tissue. The floor of the cyst was formed by necrotic pancreatic tissue. The cyst is interpreted as a pseudocyst, occurring in a pancreas that was the seat of an acute hemorrhagic pancreatitis.

O. SAPHIR.

INTERSTITIAL PNEUMONIA. L. HONECKER, Frankfurt. Ztschr. f. Path. **40**:477, 1930.

A case of interstitial pneumonia is reported in a man, aged 59. The diagnosis was lobar pneumonia involving the right lung. At autopsy, 800 cc. of a yellowish fibrinous fluid was found in the right pleural cavity. Formaldehyde was injected

into the lungs before they were opened. On section, the septums were found to be larger than normal and yellow, and they showed some pus, especially in the lower lobe. Microscopically, the septums were broad and contained dense polymorphonuclear leukocytic infiltrations. The lymph spaces were markedly dilated and filled with polymorphonuclear leukocytes. In some portions, the alveoli contained an edematous liquid, polymorphonuclear leukocytes, red blood cells and desquamated cells. There also were several abscesses. Streptococci were found in sections taken from the right lung. The opinion is expressed that streptococci are the specific cause of interstitial pneumonia.

O. SAPHIR.

MULTIPLE TUBEROUS OSTEOMAS OF THE LUNG. S. M. DERISCHANOFF, Frankfurt. Ztschr. f. Path. **40**:485, 1930.

A case of osteomas of the lung is reported in a man, aged 21. There was also a chronic endocarditis of the mitral valve with insufficiency of the valve and stenosis of its orifice. Multiple emboli were found in the branches of the pulmonary arteries. The lungs revealed many well circumscribed nodules, the size of peas, which could easily be shelled out. They were white and of bony consistency. Histologically, they consisted of typical bone structures with marrow spaces and haversian canaliculi. The author believes that the osteomas were the result of an organized pneumonia from which the patient had suffered four years previously.

O. SAPHIR.

CONTRACTION OF THE SUPRARENAL GLANDS AND ATROPHY OF THE TESTES. E. GÜNTZ, Frankfurt. Ztschr. f. Path. **40**:490, 1930.

A case of Addison's disease is described. It presented a clinical picture of a paratyphoid infection, and at autopsy showed a cytotoxic contraction of the suprarenal glands. It also showed a marked atrophy of the testes in which the interstitial cells were markedly decreased. The attempt is made to correlate the changes in the suprarenal cortex with those in the testes. The author believes that the atrophy of the testes followed the changes in the suprarenal cortex. It is possible that the hypophysis, which was not examined histologically, played a contributory rôle in causing the atrophy of the testes. As an example of a relationship between changes in the endocrine glands, an additional case of hypophyseal cachexia (Simmond's disease) is briefly reported.

O. SAPHIR.

AORTITIS THROMBOTICA. L. DESCLIN, Frankfurt. Ztschr. f. Path. **40**:520, 1930.

Seven cases of aortitis thrombotica are reported. The inflammatory process may reach the aorta from contiguous organs or tissues, or the aortitis may be the result of an acute vegetative endocarditis of the aortic valve. In the latter case, the infectious material may reach the aortic intima either by direct extension from the valvular endocardium or by an implantation on the aortic intima. It is also possible that broken, loose vegetations are carried by the blood stream directly to remote portions of the aortic intima or reach the aorta indirectly by means of the vasa vasorum and thus produce an aortitis. It is also conceivable that another primary focus of infection, aside from the endocarditis, might constitute the source of infectious emboli, which occasionally might lodge in the vasa vasorum. The author also believes that an aortitis sometimes is the primary lesion, as is an endocarditis. In the individual case, it is often impossible to establish the exact pathogenesis of the aortitis.

O. SAPHIR.

MALFORMATION OF THE BRAIN WITH HYDROCEPHALUS AND PORENCEPHALUS. I. WERTKIN, Frankfurt. Ztschr. f. Path. **40**:571, 1930.

A case of malformation of the brain is reported in a boy, aged 15 weeks. The largest portion of the cerebrum was replaced by a clear liquid. Only the lower

and posterior portions of the left hemisphere were present. The right hemisphere was almost completely converted into a saclike cavity filled with a clear liquid. The corpus callosum was not present. The anterior portions of the left hemisphere showed outspoken microgyria. In the region of the boundaries between the right temporal, occipital and parietal lobes there was a depressed area measuring from 2 to 3 mm. in diameter, from which the sulci and gyri radiated in various directions. The corpora quadragemina and the sylvian aqueduct were partly absent. The fourth ventricle was not dilated. The superior vermis of the cerebellum was not present. The branches of the vessels ran superficially and did not extend into the fissures. There were two central canals found in the region of the medulla oblongata. The hydrocephalus is explained on the basis of an atresia of the sylvian aqueduct. The various possibilities leading to these malformations are discussed.

O. Saphir.

Hemorrhagic Diathesis. P. Morowitz, Verhandl. d. deutsch. path. Gesellsch. **25**:32, 1930.

The author's conclusions as to the hemorrhagic diathesis are drawn from clinical, anatomic and experimental investigations. Essentially these conclusions are that hemorrhagic diathesis results from changes in the walls of blood vessels, either by infections or by toxic, hormonal or nervous mechanisms, and that platelets play a minor rôle.

Hemophilia.—The prolonged coagulation time in hemophilia is not sufficient to explain the hemorrhagic tendency, for animals given injections of hirudin, by which the coagulation time is prolonged, bleed only from wounds, and not spontaneously. The author believes that the disease is associated with a vasomotor disturbance. There are no definite pathologic anatomic changes in hemophilia.

Thrombocytopenia.—The belief that in thrombocytopenia the giant cells of the bone marrow are sufficient in number, but defective, does not harmonize with the fact that animals poor in platelets and fibrinogen do not show hemorrhagic manifestations. The rôle of the spleen has not yet been fully determined, since in many instances the removal of the spleen does not end the bleeding. The importance of the vascular changes has stimulated the invention of an instrument by which vascular changes and the formation of thrombi can be studied in the circulating blood. This is called a thrombometer (Jürgens).

Scurvy.—The infectious nature of scurvy following a deficiency of vitamins is considered as the probable underlying factor and not a decrease in the cement substance, as described by Aschoff and Koch, because no endothelium is found loose in the blood, while anatomic changes are found in the blood vessels.

Schönlein-Henoch Purpura.—In Schönlein-Henoch purpura, an urticarial eruption that comes out in crops is followed by hemorrhagic extravasations in these areas. This is believed to be due to a toxic action on the endothelium or to anaphylaxis (Ganzmann). The symmetry in which these lesions sometimes occur suggests a central origin by the way of the vasomotor system. From then on one has capillary dilatation, stasis, exudation and bleeding.

Sol Roy Rosenthal.

Essential Thrombopenia. Schmincke, Verhandl. d. deutsch. path. Gesellsch. **25**:50, 1930.

A case of essential thrombopenia is reported, in which the number of megakaryocytes in the bone marrow was markedly diminished, in spite of active granulopoiesis and erythropoiesis. In the spleen were giant cells derived from the reticulum cells. The giant cells are regarded as compensatory for the deficiency in the bone marrow. The underlying cause is believed to be a disturbance of the giant cells in the bone marrow (with decrease in the formation of platelets) rather than a destruction of the platelets in the spleen.

Sol Roy Rosenthal.

ANATOMIC CHANGES IN AGRANULOCYTOSIS. W. KOCH, Verhandl. d. deutsch. path. Gesellsch. **25**:53, 1930.

Agranulocytosis, first described as a clinical entity by Werner Schultz, occurs usually in women between 30 and 60 years of age. The attack is accompanied by high fever and icterus and at times by herpes. Angina sets in, which may go on to gangrene and paratonsillitis. The lymph follicles of the pharynx, the base of the tongue, the epiglottis, the larynx, the gums and the uvula enlarge and may suffer necrosis. The lymph nodes of the cervical and maxillary groups may swell. Necrotic conjunctivitis, gangrenous colpitis, vulvitis and proctitis may develop, as well as vesicles, papules and erysipeloid lesions of the skin. Diarrhea results from the intestinal ulcerations. The liver and the spleen are usually enlarged. Hemorrhagic diathesis is not common. The blood shows a marked decrease in the number of the white cells, the highest count being rarely over 1,800 per cubic millimeter. Mainly lymphocytes and monocytes are present. The blood platelets are normal. The red blood corpuscles are not reduced in number. Bacteriologic examination has yielded no specific organism. Death usually follows hemorrhagic bronchopneumonia or pulmonary gangrene. In 7,000 autopsies the author found 22 cases of agranulocytosis. Ulcerated and necrotic lesions were found involving all the mucous membranes, and in many instances thrush was present. These changes were probably all secondary. Necrotic foci, usually perivascular, were also noted in the liver, spleen and bone marrow. The striking feature is the paucity of reactive cellular elements. A few lymphocytes and monocytes are found, but no leukocytes. The etiologic factor is considered to be a cryptogenic infection, which is unrecognized because of lack of fever, or a toxin perhaps derived from the intestine. These bacteria or toxins destroy the leukocytes, which predisposes the body to secondary infection. With this complication, fever and the acute toxic state set in. The ulcerated and necrotic character of the lesions is due to the lack of response of the body, especially of the granulocytes.

SOL ROY ROSENTHAL.

ACUTE ALEUKEMIC "RETICULOSIS." K. TERPLAN, Verhandl. d. deutsch. path. Gesellsch. **25**:69, 1930.

"Reticulosis or reticulo-endotheliosis" is defined as a disease of the blood in which the reticulum cells of the liver, spleen, lymph glands and bone marrow proliferate to a marked degree. The reticulo-endothelium of the other organs may also proliferate. These changes are usually associated with a relative or absolute increase in the number of the monocytes or monoblasts (reticulo-endothelial cells) of the blood. Rarely an aleukemic form exists in which there are no characteristic changes in the blood. The author holds this to be an infectious disease, as the patients are septic, have high temperatures and die within a short period of time (eight weeks). The course is similar to that of acute leukemia. The author describes a case of an aleukemic reticulosis in a 1 year old child; a high temperature developed suddenly with generalized lymphadenopathy, anemia, hemorrhages into the skin and necrotic angina. Death occurred ten days after the onset. The blood was normal, except for the anemia. At autopsy there were a general enlargement of the lymph nodes and of the liver and pneumococcal foci in the lung. Microscopically, there was marked proliferation of the reticulo-endothelium in the lymph glands, the lung and the heart. The liver and the myocardium showed interstitial inflammatory changes. The histologic picture was very similar to that of typhoid fever because of the presence of large swollen histiocytic cells.

SOL ROY ROSENTHAL.

ON THE ORIGIN OF SO-CALLED POLYBLASTS. G. SEEMANN, Verhandl. d. deutsch. path. Gesellsch. **25**:77, 1930.

By vital staining of the loose connective tissue in rats with neutral red and janus green, the author demonstrates that the local histiocytes and fibrocytes are

two distinct types of cells. The former is plump and contains many coarse, neutral red-staining granules, while the latter is spindle-shaped with a few finely granular red elements. On injection of a peptone broth, a reaction is set up in the connective tissue, and in from three to five hours there is an infiltration by granulocytes and round cells. The latter are not lymphocytes, but monocytes or monocytoid cells, derived from the blood stream. After from six to eight hours the local histiocytes proliferate, while the local fibrocytes and endothelial cells are inactive. The author concludes that the polyblasts of Maximow have two origins: that from the local histiocytes and that from the monocytes of the blood. He does not believe that lymphocytic cells produce polyblasts, but that what Maximow called lymphoblasts were really monocytoid cells, and that the latter, not the former, are the stem cell.

SOL ROY ROSENTHAL.

Microbiology and Parasitology

ACTINOMYCOSIS STARTING AS APPENDICITIS WITH EXTENSIVE VISCERAL INVOLVEMENT. GEORGE M. ROBSON, Am. J. M. Sc. **181**:692, 1931.

Two cases of actinomycosis of the liver and lungs are reported. Both followed operations for appendicitis; in each there developed a discharging abdominal sinus, symptoms in the upper abdominal region and severe pulmonary suppuration. In one case there was a generalized dissemination by the blood stream, with additional lesions in the brain, spleen and kidneys. The diagnoses were not made during life, principally because the ray fungi were not found in the discharges.

AUTHOR'S SUMMARY.

NATIVE INFESTATION WITH DIPHYLLOBOTHRIUM LATUM (FISH TAPEWORM). I. PILOT and I. M. LEVIN, Am. J. M. Sc. **181**:710, 1931.

Five cases of native infestation by Diphyllobothrium latum are reported in children. All of our patients were Jews; twenty-one of twenty-six cases of native infestations collected from recent literature occurred in children from 3 to 15 years of age; sixteen of the twenty-one were Jews. Infestation in three of our cases occurred from sampling the raw fish while it was being prepared into "gefüllte fish." The other two probably developed from improperly cooked fish.

AUTHORS' SUMMARY.

LEPTOSPIRA ICTEROHEMORRHAGIAE IN WILD RATS ABOUT SAN FRANCISCO BAY. J. R. RIDLON, Pub. Health Rep. **46**:1, 1931.

In the kidneys of wild rats from cities on San Francisco Bay, spirochetes were found that apparently conform to the descriptions of Leptospira icterohaemorrhagiae. Guinea-pigs were inoculated with material from the kidneys of rats harboring the organisms and died, showing fever and jaundice of the eyes and skin before death. At autopsy they showed subcutaneous jaundice and hemorrhages of subcutaneous tissues and internal organs, which are the gross pathologic changes described by several writers as typical of infection with Leptospira icterohaemorrhagiae. The organisms were found in the internal organs and urine of infected guinea-pigs. Positive cultures were obtained. Guinea-pigs were infected by injection of the positive cultures. The disease was carried over in successive inoculations of guinea-pigs, both by injections of original rat material and by injections of cultures.

AUTHOR'S SUMMARY.

THE OCCURRENCE OF INTRANUCLEAR INCLUSIONS IN THE NERVE CELLS IN POLIOMYELITIS. E. W. HURST, J. Path. & Bact. **34**:331, 1931.

The bodies described are probably nuclear inclusions of the same order as those in herpetic encephalitis, Borna's disease, etc.; they are, however, much less

numerous, smaller and more difficult to stain electively than these other inclusions. They can be distinguished from the acidophilic masses normally present by their greater sharpness of outline and their more solid appearance and by the fact that under conditions leading to their appearance the acidophilic coagulum is wanting in whole or in large part. Their paucity and the limited time during which they can be found do not favor a study of their evolution, but it seems possible that they may represent a nuclear reaction to the attack of the virus. It is known that after intracerebral inoculation the virus does not reach the cord, at least in detectable amount, until immediately before the onset of paralysis (Fairbrother and Hurst, 1930); the bodies have never been found during the incubation period. They occur always in damaged cells but, on the other hand, they have not been seen in completely necrotic cells. In the poliomyelitis of monkeys, a considerable proportion of the anterior horn cells undergo complete necrosis within a few hours of the onset and do not necessarily pass through a phase of degeneration in which the bodies are present; indeed, the latter may develop only in cells possessing rather more power of resistance to the virus and which consequently degenerate more slowly. However this may be, they have not been seen in markedly degenerate cells in the cords of rabid monkeys or in a series of other pathologic and normal tissues. Similar bodies were present in the only human case examined, and as far as this limited evidence goes, they appear to be characteristic of the disease.

AUTHOR'S SUMMARY.

SILENT TYPHUS INFECTION. W. BARYKINE, S. MINERVINE and A. KOMPANÉEZ, Arch. Inst. Pasteur de Tunis **19**:422, 1930.

Epidemiologically, silent typhus infection is as important as the type in which symptoms appear. The silent infection may be seen in those who have and in those who have not been previously infected. This type of the disease may be suspected from an increase in the Weil-Felix reaction, but may be proved only by inoculating guinea-pigs with the blood of the patient.

INOCULATION WITH BACTERIUM GRANULOSIS. CHARLES WEISS, Arch. Inst. Pasteur de Tunis **19**:433, 1930.

Two strains apparently of Bacterium granulosis were isolated by Noguchi's methods and inoculated into the eyes of monkeys. In two weeks flaky granulations were noted, and conjunctivitis with marked redness and edema of the eyelids. This condition was maintained for several weeks, after which recovery occurred slowly. The original Noguchi strain was inoculated into three human volunteers and one monkey, but no reaction was observed.

M. S. MARSHALL.

SPONTANEOUS TUBERCULOSIS IN DOMESTIC ANIMALS WITH REFERENCE TO RANKE'S TEACHING. K. NIEBERLE, Beitr. z. Klin. d. Tuberk. **75**:179, 1930.

The essential thesis of this paper is that Ranke's teachings are fully confirmed by studies on the spontaneous tuberculosis of domesticated animals.

MAX PINNER.

STUDIES ON TUBERCULOUS LIQUEFACTION. W. PAGEL, Beitr. z. Klin. d. Tuberk. **76**:414, 1931.

Tuberculous liquefaction can start without the aid of cells and inflammatory processes; it may be caused by hydration of the focus or by the so-called liquor diapedesis (Ricker) from blood vessels. In the incipient stage of liquefaction, macrophages and histiocytes, and not polymorphonuclears, are prominent. Tubercle bacilli, in some instances, become visible only after the incipient stage is passed. Liquefaction and caseation are separate processes; the former supersedes the

latter as a new development. Secondary organisms do not play a causative rôle in liquefaction. In experiments on animals it was shown that specific allergy is an essential factor in the causation of liquefaction. By irradiating a focus of first infection it is possible to simulate to a lesser degree the allergic tissue reaction. Three types of liquefaction may be distinguished: (1) liquefaction in attenuated and prolonged infection, (2) allergic liquefaction and (3) nonspecifically stimulated liquefaction.

MAX PINNER.

THE LATE TUBERCULOUS PRIMARY COMPLEX IN ADULTS. E. RAGNATTI, Beitr. z. Klin. d. Tuberk. **76**:459, 1931.

A tuberculous primary infection may be acquired at any age; its occurrence after puberty, however, is rare. In 4,000 consecutive autopsies performed on adults, there were 36 in which a recent primary complex was found. In 72 per cent it was an aerogenous focus; in the remaining 28 per cent it was an intestinal focus. In all aerogenous foci the regional lymph nodes were characteristically involved. In every detail, primary complexes arising in adults have the same characteristics as those arising in children.

MAX PINNER.

TUBERCLE BACILLI IN THE BLOOD IN LARYNGEAL TUBERCULOSIS. E. WESSELY and E. LÖWENSTEIN, Beitr. z. Klin. d. Tuberk. **76**:647, 1931.

The recent method of Löwenstein for the isolation of tubercle bacilli from the blood was used. Of forty-seven patients with laryngeal tuberculosis, twenty-six yielded tubercle bacilli from the blood (only one examination for each patient).

MAX PINNER.

CHEMICAL DIFFERENTIATION OF HEMOLYTIC AND NONHEMOLYTIC STREPTOCOCCI. L. AVILÉS, Ztschr. f. Immunitätsforch. u. exper. Therap. **69**:433, 1931.

By means of the berlin blue reaction, it could be shown that hemolysis by certain streptococci may be due to destruction of hemoglobin in the bodies of the streptococci, because the colony itself became blue, while the hemolytic zone was unchanged. In the case of nonhemolytic streptococci, on the other hand, the colony itself remained white, while a blue zone due to the liberation of inorganic iron developed around the colony. This difference was marked on chocolate agar plates, more so on plates made with human blood than on plates made with beef or sheep blood.

GAS-FORMING VARIANTS OF THE TYPHOID BACILLUS. C. SONNENSCHEIN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **69**:454, 1931.

The claims that the typhoid bacillus may develop gas-forming variants could not be substantiated.

PERORAL INFECTION OF GUINEA-PIGS IN TUBERCLE BACILLI. J. ORSKOV and K. A. JENSEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **70**:146, 1931.

Following oral introduction of virulent tubercle bacilli, infection develops step by step by way of the lymphatics and lymph nodes. The first localization is in the regional lymph nodes.

CULTIVATION OF TUBERCLE BACILLI FROM THE BLOOD ACCORDING TO LÖWENSTEIN. A. FISCHER, Ztschr. f. Tuberk. **58**:331, 1930.

Seventy-seven specimens of blood from tuberculous patients in various stages of the disease were cultured, according to the recently published technic of Löwenstein, and from thirty-one of them tubercle bacilli were obtained in pure culture.

MAX PINNER.

THE FILTRABLE ELEMENTS OF THE TUBERCULOSIS VIRUS. A. CALMETTE and J. VALTIS, Ztschr. f. Tuberk. **58**:402, 1930.

Filtrates from young cultures of the tubercle bacillus, from tuberculous organs or from excreta cause, after subcutaneous injection, lymphoglandular swellings in guinea-pigs. The involved nodes that do not show tuberculous lesions contain few acid-fast rods and granules (from three days to eight weeks after the injection). Such nodes produce, after a few direct animal transfers, typical tuberculous lesions. These filtrable elements are capable of passing through the placenta both in experimental animals and in human beings. The filtrable virus can be cultivated in vitro, and under this condition its development into typical tubercle bacilli can be observed. Transplants of the virus have been unsuccessful as yet. The filtrable virus can be demonstrated most readily by injecting it directly into lymph nodes or into the spinal canal. The authors conclude that the disease tuberculosis, characterized by specific changes in the tissues and by the presence of tubercle bacilli, is only the chronic end-stage of an infection caused by the filtrable virus, which per se does not produce tubercles, but which may exert marked toxic influences. This virus is considered the etiologic agent in such diseases as purpura rheumatica, erythema nodosum, sarcoids, papulonecrotic tuberculids, typhobacillosis Landouzy and the "granulémie" (i. e., miliary tuberculosis). It is suggested that all diseases caused by the filtrable virus be called "prebacillary granulemia," and those caused by true tubercle bacilli, "bacillosis."

MAX PINNER.

SECONDARY COLONIES, AUTOLYSIS AND LYSIS IN CULTURES OF TUBERCLE BACILLUS. E. A. SCHNIEDER, Ztschr. f. Tuberk. **59**:18, 1930.

In culturing tubercle bacilli the formation of secondary colonies and lytic phenomena can be observed in a similar way as with other micro-organisms. Secondary colonies and lysis appear to be closely related occurrences; they are both enhanced by unfavorable mediums.

MAX PINNER.

A TUBERCULOUS PRIMARY FOCUS WITH ATYPICAL HISTOLOGIC STRUCTURE. M. PINNER, Ztschr. f. Tuberk. **59**:130, 1930.

A primary tuberculous focus in the lung is described that showed, in addition to the usual type of encapsulation, the formation of a diffuse intra-alveolar connective tissue.

MAX PINNER.

EXPERIMENTAL TUBERCULOSIS. W. A. LUBARSKI and A. F. KORSCHINSKAJA, Ztschr. f. Tuberk. **59**:252, 1931.

Following intravenous injection of tubercle bacilli into the rabbit, the lungs show the most massive involvement. After subcutaneous injection of tubercle bacilli into the guinea-pig, the lungs and spleen are mostly involved. The number of tubercle bacilli is largest in the lungs, and considerably less in the spleen and liver. Tubercle bacilli must be destroyed in the organs. This destruction is most marked in the liver. The organs of white mice have the greatest capacity to destroy tubercle bacilli. In pure cultures from organs of infected animals, three different types of colonies are observed.

MAX PINNER.

HOHN'S MEDIUM AND THICK SEEDING OF SEDIMENT. L. FRANKL, Ztschr. f. Tuberk. **59**:262, 1931.

When the sediment is seeded thickly on Hohn's medium, tubercle bacilli are demonstrable in about 50 per cent of the positive cases within the first few days, while after thin seeding the first colonies become visible only after from eight to thirty days.

MAX PINNER.

THE SIGNIFICANCE OF THE SILICIC ACID CONTENT OF THE BLOOD IN TUBERCULOUS PATIENTS. R. H. KRANZFELDER, Ztschr. f. Tuberk. **59**:398, 1931.

The amount of silicic acid in normal persons (twenty-three) is 16 mg. per hundred cubic centimeter of blood; within four months the variations observed were less than 4 per cent. In tuberculous patients the values are, on the average, 18 per cent higher. The silicic acid content of the blood increases readily after administration by inhalation; it may increase more than the amount administered explains. Patients with high silicic acid content (without previous administrations) have, in general, a poorer prognosis than patients with lower values.

MAX PINNER.

THE RELATION OF CELL GRANULES TO PHAGOCYTOSIS OF BACTERIA. H. HAZATO, Jap. J. Exper. Med. **9**:67, 1931.

Bacteria taken up and digested in histiocytes are regularly included in neutral red granules or vacuoles, in which they undergo digestion. When neutral red granules or vacuoles have previously been impregnated with sepia or carmine the majority of phagocytosed bacteria are capable of finding their way into the preformed sepia or carmine granules or vacuoles to be digested therein, but never into degenerative ones. When a histiocyte phagocytoses and digests bacteria, the neutral red granules or vacuoles, as well as the fat-droplets or lipin of the cell, show a gradual increase in number and in size, and the cell itself is correspondingly enlarged. With the distinct increase in number and in size of the neutral red granules or vacuoles, there is a gradual decrease of the number of mitochondria. At this stage the mitochondria of the histiocyte show a greater degree of osmic reaction than normal.

AUTHOR'S SUMMARY.

RICKETTSIA ORIENTALIS, THE CAUSE OF TSUTSUGAMUSHI DISEASE. M. NAGAYO and others, Jap. J. Exper. Med. **9**:87, 1931.

When the virus of Tsutsugamushi disease is introduced into the anterior chamber of the eye in rabbits and other animals it causes a peculiar lesion, one of the characteristics of which is the accumulation of minute organisms in the endothelial cells of Descemet's membrane. These organisms correspond to those found in patients suffering from the disease and in experimentally infected animals. The infected endothelial cells carry the infection with them when introduced into other animals. In cultures of the cells in vitro, the micro-organism, which is designated Rickettsia orientalis, proliferates freely. The article is illustrated with forty-seven figures, many in colors.

TOXIN OF THE SHIGA BACILLUS. H. KAWAMURA, Kitasato Arch. f. Exper. Med. **8**:1, 1931.

The specific dysentery toxin is produced within the bacillary bodies and released into the medium after the bacilli are dead. It is not a secreted toxin. The toxin weakens after a short time. The combining power of the toxin and the antitoxin increases with the increase in toxicity after the death of the bacilli and decreases as the toxin weakens. The toxin does not produce a true toxoid. A strong toxin can be developed in a peptone-free synthetic medium. A 0.5 per cent formaldehyde solution weakens the toxin. The bacilli treated with formaldehyde retain their antigenic properties, but the autolysates treated with formaldehyde have very weak antigenic power. When treated with toluene or phenol, the old preparations show no decrease in toxicity, but have little antigenic power. Toxin treated by heat at 80 C. or above and by hydrogen dioxide loses both toxicity and antigenic power.

EDNA DELVES.

THE ETIOLOGY AND PATHOGENESIS OF ACTINOMYCOSIS. CARL NAESLUND, Acta path. et microbiol. Scandinav., Supp. 6, 1931.

The main outcome of the work recorded in the German language in this monograph of 150 pages is a well supported proposal to simplify and stabilize the nomenclature of actinomycosis and its agents. It is proposed to divide the pathogenic ray fungi (Actinomyces, Streptothrix, Nocardia, etc.) into two groups, the anaerobic and aerobic, and to call the first group Actinomyces A and the corresponding diseases actinomycosis A, while the second group is called Actinomyces B and the corresponding diseases actinomycosis B.

Immunology

THE ANTIGENIC PECULIARITIES OF STRAINS OF SPIROCHAETA PALLIDUM. W. M. ARISTOUSKY and A. J. WSOROW, Ztschr. f. Immunitätsforsch. u. exper. Therap. **69**:351, 1931.

Spirochaeta pallidum contains species-specific and type-specific antigens which, so to speak, compete with one another in their action on the organism.

ANAPHYLACTIC SHOCK FROM INTRACEREBRAL INJECTION OF ANTIGEN. A. SCHWARZMANN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **69**:379, 1931.

The shock after intracerebral injection of antigen into sensitized guinea-pigs is due to the passage of the antigen into the blood. The mechanism of the shock is the same as in intravenous injection of the antigen; it requires about four times as much antigen to produce shock on intracerebral injection of antigen as on intravenous.

PROTECTIVE INOCULATION AGAINST EPIDEMIC POLIOMYELITIS. R. KRAUS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **69**:424, 1931.

Monkeys can be protected against experimental poliomyelitis by injection of phenolvirus, which is obtained by adding from 1 to 1.5 per cent of phenol to emulsions of poliomyelitic virus and filtering through paper. The phenol kills the virus, or greatly reduces its virulence, without destroying its antigenic properties.

THE HETEROGENETIC ANTIGEN OF THE SHIGA BACILLUS. K. MEYER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **69**:499, 1931.

The heterogenetic antigen is more or less firmly united with the specific carbohydrate.

EFFECTS OF OTHER BACTERIA ON THE RESISTANCE TO INFECTION WITH TYPHOID BACILLI. S. NUKADA and S. ARIFUKU, Ztschr. f. Immunitätsforsch. u. exper. Therap. **70**:1, 1931.

In guinea-pigs immunization with certain bacteria, e. g., pneumococci, increases the resistance to fatal infection with typhoid bacilli, owing perhaps to alterations in the cells of the tissues.

THE ANTIGENS OF THE SHIGA BACILLUS. M. SISLER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **70**:48, 1931.

The carbohydrate from the Shiga bacillus acts as antigen only when it contains human blood antigen as well as the Forssman antigen. Such a carbohydrate reacts only with serum against the Shiga bacillus that in addition to protein antibodies also contains heterogenetic antibodies.

FROM AUTHOR'S SUMMARY.

THE RÔLE OF ALCOHOL-SOLUBLE CONSTITUENTS OF MILK IN ITS SEROLOGIC REACTIONS. M. DEHIO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **70**: 58, 1931.

By immunizing rabbits with extract of milk in 50 per cent alcohol, an antiserum was developed that reacted with the homologous antigen as well as with the native milk. Following treatment of rabbits with extract of milk, in 96 per cent alcohol antibodies did not develop, at least not regularly. Aqueous and alcoholic solutions of sodium caseinate reacted with antimilk serum. The action of antimilk or anticasein serum on heterologous milk may depend, at least in part, on the alcohol-soluble constituents in milk.

FROM AUTHOR'S SUMMARY.

ACTION OF LYSOZYME ON MICROCOCCUS LYSODEIKTICUS. O. ANDERSEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **70**:90, 1931.

This article describes the results of studies of the action of lysozyme from egg-white on Micrococcus lysodeikticus, a sarcina. Pathogenic bacteria and animal cells are not subject to the action of lysozym.

THE ACTION OF B C G. K. A. JENSEN and J. ORSKOV, Ztschr. f. Immunitätsforsch. u. exper. Therap. **70**:155, 1931.

Following the oral introduction of B C G into guinea-pigs there was no general invasion of the lymphatics by tubercle bacilli, but localization in the most peripheral parts of the regional lymphatic system. Does this process suffice to produce a fairly constant immunity? The results in animals indicate that the answer to this question is no.

THE PRODUCTION AND PURIFICATION OF TUBERCULIN. E. MASCHMANN and E. KÜSTER, Ztschr. f. Tuberk. **59**:225, 1931.

Sauton's protein-free medium is very suitable for the production of a potent tuberculin. Tuberculin prepared with Sauton's medium shows reactions that are characteristic for proteins and carbohydrates. The specifically active substance diffuses easily through animal membranes. By acidifying the tuberculin before or after dialysis, high molecular proteins are removed without diminishing noticeably the potency of the tuberculin. The specific substance is easily adsorbed by kaolin, particularly in acid reaction. The adsorbed tuberculin can be liberated from the kaolin by very dilute ammonia; the yield is good and constant. By this process the strength of the tuberculin is increased twenty-one times, compared with the original tuberculin. The finished products have a positive biuret and ninhydrin reaction; some show a positive Millon reaction; none show a tryptophan or Molisch reaction. For the specific action the following constituents are unnecessary: the carbohydrates, those proteins that are precipitated in acid solution and those proteins that produce a positive Millon and tryptophan reaction. It must be determined whether the specific factor is a polypeptide or whether it belongs in another chemical class. A pure carbohydrate was isolated that is identical with glycogen.

MAX PINNER.

THE PROTECTIVE ACTION OF THE B C G VACCINE. A. I. TOGUNOWA, W. N. ARCHANGELSKY and S. L. BAIDAKOWA, Ztschr. f. Tuberk. **59**:425, 1931.

Guinea-pigs and rabbits given subcutaneous injections of B C G vaccine at once develop a relative immunity against a later subcutaneous or intra-ocular infection with virulent bacilli. The duration of this immunity is dependent on the animal species and on technical detail.

MAX PINNER.

Tumors

THE ORIGIN AND NATURE OF MENINGEAL TUMORS. P. BAILEY and P. C. BUCY, Am. J. Cancer **15**:15, 1931.

The authors are of the opinion that meningeal tumors, whatever their origin, are of the nature of connective tissue and are not gliomatous. This is in disaccord with the opinion of those who claim that the leptomeninx is of neuro-ectodermal origin. They found at least nine distinct histologic types of tumors arising in the meninges. The relationship of these tumors to each other is represented by them in a diagram. They further believe that the fibroblast is usually not the typical cell of these tumors as it is claimed by Mallory and Penfield.

B. M. FRIED.

MALIGNANT MIXED TUMORS OF THE THYROID GLAND WITH SKELETAL MUSCLE FIBERS. EDWIN F. HIRSCH, Am. J. Cancer **15**:55, 1931.

A malignant tumor of the thyroid gland and its metastasis in many of the visceral tissues of the body contained embryonal skeletal muscle fibers, undifferentiated cells and large fused-cell aggregates. Some of the fused-cell aggregates contained hyaline masses with the staining qualities of thyroid colloid. These cell masses, however, were not identified conclusively as abortive thyroid acini, although some resembled such structures closely.

AUTHOR'S SUMMARY.

A STATISTICAL STUDY OF THE OCCURRENCE OF SPONTANEOUS TUMORS IN A LARGE COLONY OF RATS. M. R. CURTIS, F. D. BULLOCK and W. F. DUNNING, Am. J. Cancer **15**:67, 1931.

The article represents a detailed statistical analysis of a large rat colony in respect to the occurrence of spontaneous neoplasms.

B. M. FRIED.

EMBRYONAL NEPHROMA IN THE CHICKEN. F. D. MCKENNEY, Am. J. Cancer **15**:122, 1931.

The two rare tumors reported belong to the group of neoplasms which, when found in human beings, are known as Wilms' tumors. The tumors were growing within the kidney without having any capsule and not being attached to the spinal column. No metastasis could be demonstrated, although the histologic picture of the new growths was that of malignant tumors.

B. M. FRIED.

BIOCHEMICAL STUDIES OF MALIGNANT CONDITIONS. JOSEPH H. ROE and HELEN M. DYER, Am. J. Cancer **15**:125, 1931.

Dextrose tolerance test showed a lowered carbohydrate tolerance in a fowl bearing the Rous sarcoma no. 1. The reduced carbohydrate tolerance is probably secondary to changes produced in the organs regulating carbohydrate metabolism, either by metastases or by toxemia due to the malignant growth.

The glycogenolytic activity of the blood of hens in which the Rous sarcoma no. 1 had developed showed no variation from that of normal controls.

The blood of a fowl bearing the Rous sarcoma no. 1 has a glycolytic activity about twice as great as that of a normal fowl. This is a primary change resulting apparently from an overflow into the blood stream of the glycolytic enzyme, which Warburg showed to be in tumor tissue in greatly increased amounts.

An examination of the glycolytic activity of the blood of sixteen patients with malignant tumors gave values within normal limits. The determination of the glycolytic activity of the blood is, therefore, of no clinical value in studying

malignant conditions. The discrepancy between the glycolytic activity of the blood of hens with Rous sarcoma no. 1 and that of the blood of patients with malignant conditions is probably due to the greater ratio of tumor tissue to normal tissue in the fowl with the sarcoma.

AUTHORS' SUMMARY.

THE INFLUENCE OF EXTRACTS OF SUPRARENAL CORTEX ON THE GROWTH OF CARCINOMA, SARCOMA AND MELANOMA IN ANIMALS. K. SUGIURA, Am. J. Cancer **15**:129, 1931.

The toxic action of extracts of suprarenal cortex of the sheep and cattle on the Flexner-Jobling rat carcinoma, the Sugiura rat sarcoma, the Bashford mouse carcinoma no. 63, a transplantable mouse melanoma and the Rous chicken sarcoma has been investigated. The author found that single or repeated subcutaneous or intramuscular injections of an alcoholic, ether, aqueous or glycerin extract of the cortical tissues has no apparent influence on the growth of transplanted tumors. Injection of choline solution or feeding with sodium nitrite chemicals known to cause a prompt fall of blood pressure failed to show any inhibiting effect on tumor growth. Extracts from the adenomatous growths of sheep suprarenal glands which are almost completely free from medullary tissue, produced no effect on the growth of neoplasms. The repeated subcutaneous injection of extracts of the suprarenal glands of sheep, of immune and normal young rats and of suckling rats does not affect the growth of rat and mouse carcinoma. Finally, fresh sheep suprarenal cortex or its extract failed to destroy cancer cells in vitro.

B. M. FRIED.

OCCUPATIONAL NEOPLASTIC DISEASES. C. D. HAAGENSEN, Am. J. Cancer **15**:641, 1931.

Haagensen, in accord with other workers, found a high incidence of skin cancer among workers in gas works and petroleum refineries. He also found that occupational exposure to lubricating oil, as in the case of mechanics and machinists, predisposes to cancer of the external genitalia — a predilection as to anatomic site which seems best explained by the uncleanliness of these organs common in laborers. Likewise, an excess of cutaneous cancer among outdoor workers affecting the face and the lip shows the importance of sunlight in the etiology of cutaneous cancer.

Metastases occurred in 63 per cent of cases of occupational roentgen cancer, which suggests that this type of cancer is more malignant than some workers have reported it to be. The author could find no association between cancer of the lung and bladder with occupation. In general, occupational exposure is most likely only one of the multiple etiologic factors of cancer.

B. M. FRIED.

THE SUPRARENAL AND TUMOR GROWTH. W. H. WOGLOM, Am. J. Cancer **15**:704, 1931.

The suprarenal glands of rabbits that have been inoculated with carcinoma "63" or sarcoma "180" have no demonstrable inhibitory effect on the growth of these tumors in mice.

B. M. FRIED.

THE INFLUENCE OF AN AQUEOUS EXTRACT OF SUPRARENAL CORTEX ON THE GROWTH OF CARCINOMA, SARCOMA AND MELANOMA IN ANIMALS. K. SUGIURA, Am. J. Cancer **15**:707, 1931.

Repeated subcutaneous or intramuscular injections of aqueous extracts of sheep suprarenal cortex, which were prepared according to the method described by Coffey and Humber, failed to retard or inhibit the proliferation of carcinoma, sarcoma or melanoma cells. These tumors, whether young or old, grew normally

and killed the hosts in the usual manner in spite of the administration of large or small doses of cortical extracts. There was no evidence that injection of the aqueous extracts of cortical tissues accelerated the growth of these transplantable tumors, nor did the injection of the aqueous extracts of sheep suprarenal cortex have any appreciable effect in checking the development of metastases in visceral organs of sarcoma-bearing chickens. Metastatic growths were observed as frequently in the treated as in the untreated animals.

B. M. FRIED.

IMMUNITY TO TRANSPLANTABLE RAT TUMORS. RAYMOND E. GARDNER and ROSCOE R. HYDE, Am. J. Hyg. **13**:649, 1931.

Groups of rats from a stock susceptible to transplants of a spindle cell sarcoma (Walker's tumor no. 1) and of a carcinoma (Walker's tumor no. 256) were given preliminary treatment with a variety of substances before tumor inoculation. Fresh whole chicken blood given subcutaneously exerted a definite influence in preventing the growth of both sarcoma and carcinoma transplants. Nonhemolytic heat-dried whole chicken blood was successful in a smaller proportion of cases of both sarcoma and carcinoma. Whole dried chicken blood produced no resistance to the growth of either of the tumors. Feeding of whole chicken blood also produced no effect. A larger proportion of animals inoculated subcutaneously with blood-free chicken embryo skin failed to develop sarcoma than did the controls, but this was not so in the case of carcinoma. A similar difference was noted when vaccine virus was used. Vaccine virus was not recovered after inoculation into either sarcoma or carcinoma from nine to fourteen days later, although the virus is recoverable from normal tissue. The treatment of rats with dried carcinomatous tissue produced no resistance to subsequent inoculations of the same tumor.

Rats of susceptible stock proved refractory to sarcoma transplants were resistant to carcinoma transplants in 46 per cent of cases. No increase in the susceptibility of rats naturally resistant to subcutaneous implantations of sarcoma could be noted after making implantations in the brain.

In general, when any of the treatments produced fewer instances of tumor development than occurred in the corresponding control group, there was a retardation of tumor development in those treated animals in which tumor growth did take place.

P. H. GUINAND.

DOES CARCINOMA OF THE DUODENUM EVER ARISE FROM DUODENAL ULCERS? J. WILLIAM HINTON, Am. J. M. Sc. **181**:843, 1931.

During the years 1928, 1929 and 1930, 324 ulcers were observed at the gastroenterologic clinic of the fourth medical and surgical divisions of Bellevue Hospital. Of this number, there were 269 duodenal lesions, 34 gastric, 14 pyloric and 6 double ulcers, meaning a lesion in the stomach and duodenum. With the marked frequency of duodenal ulcers, it is difficult to explain why these cases do not undergo carcinomatous degeneration. Primary carcinoma of the duodenum is occasionally seen. Clinically, one can disregard the possibility of a duodenal ulcer ever taking on malignant degeneration.

AUTHOR'S SUMMARY.

PRIMARY CARCINOMA OF THE DUODENUM. JACOB MEYER and DAVID H. ROSENBERG, Arch. Int. Med. **47**:917, 1931.

Four cases of primary carcinoma of the duodenum are reported. Emphasis is placed on the difficulty of clinical recognition and of the necessity of carefully studying, clinically as well as histologically, all cases of duodenal stenosis. A critical analysis may reveal a greater incidence of primary duodenal carcinomas.

AUTHORS' SUMMARY.

PINEALOMAS. JOSEPH H. GLOBUS and SAMUEL SILBERT, Arch. Neurol. & Psychiat. **25**:937, 1931.

As the structure of tumors in the region of the pineal body (pinealomas) varies greatly, Globus and Silbert attempted to trace the formation of these tumors to embryonal rests and thus "find a basic morphologic pattern that would link all tumors in the pineal region, no matter how divergent their gross or histologic appearance." They studied pineal bodies of nine fetuses of various ages (from 5½ months to 8½ months), of eighteen infants from 1 to 24 days old, of twenty-five infants and young children between the ages of 1 month and 2½ years, of twenty-two older persons ranging up to the age of 72 years, and seven tumors; a total number of seventy-seven pineal bodies was studied. Among the several methods used were those of Bielschowsky, Cajal and Hortega's modification by Globus. In all tumors (except one), some phase of the basic pattern common to the developing pineal body during the first four months of postnatal life was found. The pattern consisted of streams of small deeply staining cells that broke up the pineal territory into irregular circles or squares. In the latter, large cells were found that stained less densely and assumed the character of parenchymal cells. The small cells were in some areas assuming the character of connective tissue. The glandular structure of the pineal body seemed unquestionable, especially about the sixth month of the intra-uterine life. In some cases the pattern was less typical. Glial or nerve elements did not play any rôle in the structure of the pineal body. The histologic nucleus of various types of tumors of the pineal region is furnished by the microscopic appearance of the pineal body in the third or fourth month of postnatal life.

GEORGE B. HASSIN.

CALCIFICATION IN GLIOMAS, CLEMENT B. MASSON, Bull. Neurol. Inst., New York **1**:314, 1931.

One hundred and thirty-one gliomas showed on roentgen examination calcification in 12.97 per cent of patients. From the character and the distribution of the shadows seen in x-ray films, it is seldom possible to differentiate between the various types of glioma, an astrocytoma from a spongioblastoma, for instance, nor is it possible to differentiate the gliomas from the meningiomas. Calcification is not rare in rapidly growing, malignant gliomas (spongioblastomas) and the area of calcification may be limited to a part of the tumor, of which the center is already involved.

GEORGE B. HASSIN.

DERMOID OVERLYING THE CEREBELLAR VERMIS WITH A REVIEW OF THE LITERATURE ON INTRACRANIAL DERMOIDS. SAMUEL BROCK and DOROTHY A. KLENKE, Bull. Neurol. Inst., New York **1**:328, 1931.

This type of tumor has no characteristic signs or symptoms. The diagnosis is made by roentgenogram, which in the authors' case showed the tumor in the posterior fossa, overlying the vermis and separating the two cerebellar hemispheres. The tumor was cystic and contained many long, dark hairs and numerous calcareous plaques which varied in size from that of a millet seed to that of a peanut. Intracranial dermoids are rare.

GEORGE B. HASSIN.

ROENTGEN CARCINOMA AND SARCOMA. F. W. MULSOW, J. A. M. A. **96**:2030, 1931.

The development of sarcoma on one finger and a year later of carcinoma on another finger is reported in the case of a physician, aged 57 years, who had been repeatedly exposed to roentgen rays for about twelve years.

THE EFFECT OF CERTAIN INTERNAL SECRETIONS ON THE GROWTH OF CANCER. F. BISCHOFF, L. C. MAXWELL and H. J. ULLMAN, J. Biol. Chem. **92**:80, 1931.

The rate of growth of a sarcoma transplanted into rats after adrenalectomy and castration was not significantly different from the growth observed in normal rats. Transplants of the sarcoma into splenectomized rats appeared to increase in size three times as rapidly as in normal rats. Splenectomy and adrenalectomy were without effect on the growth rate of subsequently transplanted carcinomas. Applications of x-rays to the head (for destruction of the pituitary body) were followed by a retardation of the rate of tumor growth. Injections of synthalin and thyroxin were without effect.

ARTHUR LOCKE.

EXPERIMENTAL TAR TUMORS IN THE WHITE RAT. A. F. WATSON, J. Path. & Bact. **34**:301, 1931.

Skin tumors were obtained in two male and two female rats from an original litter of six males and five females by means of the periodical treatment of the skin with a gas works coal tar, each application of tar being preceded by treatment of the skin with a petroleum ether extract of rat tissues. Under the particular experimental conditions adopted, the tumors appeared during the period from the 415th to the 551st day after the first application of tar. The tumors of the males were squamous cell carcinomas, and once initiated grew vigorously, one animal showing extensive metastases in the lungs, both kidneys and the pleural cavity. Inoculations of the tumor tissue from these animals into normal rats were unsuccessful. The tumors of the females grew less vigorously, and in only one case could any histologic evidence of local infiltrative growth be obtained.

AUTHOR'S SUMMARY.

PRIMARY CARCINOMA OF BRONCHUS. E. E. ATKIN, J. Path. & Bact. **34**:343, 1931.

The observations in this paper are based on autopsies in eighty cases of primary bronchial carcinoma in men and thirteen women. The mean age of the male patients with primary carcinoma of the bronchus is higher when it is a squamous cell growth than when the cells are of other types, the respective mean ages being 51 and 43.3 years in the present series. Carcinoma does not appear to arise in one bronchus more frequently than in the other. The order of frequency of occurrence of metastases in the abdominal organs was liver, pancreas, kidney, suprarenals and spleen. In about half the cases no metastases at all were found. The squamous cell carcinomas exhibit a great tendency to necrosis and liquefaction. All the cases of definite cavitation found post mortem in the growth were in squamous cell tumors. The possibility that secondary deposits in the opposite lung are due to aspiration via the bronchus is suggested. All such metastases in the present series occurred in cases of squamous cell carcinoma with a marked tendency to liquefaction.

AUTHOR'S SUMMARY.

MALIGNANT PHAEOCHROMOCYTOMA OF THE ADRENALS. E. S. J. KING, J. Path. & Bact. **34**:447, 1931.

A hitherto undescribed form of pheochromocytoma of the suprarenals is reported. The growths were solid and bilateral. Cells of two types were present: typical pheochrome cells and anaplastic cells not giving the typical chrome stain, but showing all transitions from the typical cells. Multiple metastases were present in the liver, lungs, bowel, bones, skin and aortic glands. The structure of most of these was the same as the anaplastic areas of the suprarenals, but some showed both this and typical pheochrome tissue.

AUTHOR'S SUMMARY.

ANGIORETICULO-XANTHOMA OF THE RETINA (VON HIPPEL AND LINDAU DISEASE). LOUIS BERGER and ARTHUR VALLÉE, Ann. d'anat. path. **8**:313, 1931.

The diseases described by Hippel, angiomatosis of the retina, and that described by Lindau, angiomatosis of the cerebellum and of the medulla, are the same condition with a different localization.

Berger and Vallée describe in detail an angiomatous tumor of the retina which they regard as originating from the "reticulo-endothelium" and in which a large part of the new growth had undergone a xanthomatous transformation. They, therefore, designate the tumor as angioreticulo-xanthoma. The angioma in their case varied from that of Hippel in that it invaded all the elements of the eye. The reticular nature of the growth was evidenced by the marked development of a reticulum impregnated with silver nitrate and by the abundance of intercapillary cells which were undergoing xanthomatous, reticular and "pseudo-gaucherian" transformation. The neuroglial proliferation which often accompanies the angioreticulomas does not represent an essential part of the tumor, being nothing other than a reaction to the growth. The disease occurred in a girl 12 years old. The tumor, which was diagnosed by the clinician as a glioma of the retina, was removed, and after fifteen months there was no recurrence of the disease.

The article is accompanied by eleven good drawings, five of which are in colors. The bibliography is complete.

B. M. FRIED.

TISSUE ANOMALIES AND THEIR RELATIONSHIP TO SOME TUMORS OF THE OVARIES. R. MEYER, Arch. f. Gynäk. **145**:2, 1931.

Thirty-two photomicrographs are given, illustrating seminomas, aplasia of the ovary, granuloso-cell tumors, tubular adenomas, paraganglioneuromas and tumors derived from suprarenal ovarian rests ("hypernephoid tumors"). Emphasis is laid on the origin of ovarian neoplasms from ebryonal dysplasias, either from incomplete formation of certain tissue elements or from their lack of proper retrogression. Tissue anomalies present in the ovaries of the cow and anthropoid apes are reported, and their significance in connection with the formation of certain tumors in man are discussed.

LAWRENCE PARSONS.

TUMORS DEVELOPING AFTER X-RAY TREATMENT OF BONE AND JOINT TUBERCULOSIS. H. KÜTTNER, Arch. f. klin. Chir. **164**:5, 1931.

Küttner's interest in this subject was aroused by the observation of two cases in three years. One of his patients was a girl, aged 11 years, who, at the age of 5 years, had a tuberculosis of the knee for which she received some stretching operations and a total of five skin units of x-ray therapy in twelve sittings. The amputated femur was involved in a polymorphocellular sarcoma, and recurrence followed in the stump. Ten additional case reports, from the German literature, are cited to strengthen the theory that the roentgen irradiation is the most plausible factor to which to ascribe the development of such tumors, although trauma and the tumor-producing stimulus of tuberculosis are also considered. The tumors noted in the eleven cases were spindle cell sarcomas (two), polymorphonuclear cell sarcomas (six), giant cell sarcoma (one) and enchondromas (two). Six males and five females were in the series; eight had tuberculosis of the knee, two of the elbow and one of the hand. Ten patients were from 11 to 23 years old, while one (the patient with the hand involvement) was 39. An interval of from two to eleven years had elapsed between the time of healing of the tuberculous lesion and the appearance of the malignant tumor. The original diagnosis of tuberculosis seemed correct in all instances. One patient who had both a knee and hip involvement received x-ray treatment for his knee but not for his hip, and subsequently a sarcoma of the knee developed. Straying from his subject to cite evidence, Küttner brings in sarcomas and carcinomas of the face which develop on lupus following treatment with the x-rays.

A second type of tumor reported is a myeloma in a man 44 years old who had numerous x-ray treatments for a thoracic tuberculous spondylitis over a period of eighteen years.

GEORGE RUKSTINAT.

A CASE OF A TERATOID TUMOR OF THE HYPOPHYSEAL REGION. G. J. DU MARCHIE SARVAAS, Frankfurt. Ztschr. f. Path. **40**:210, 1930.

In a boy, 12 years of age, a tumor was found in the region of the sella turcica. The tumor, which extended through the nares to the outside, had led to an open communication between the orbit, the nasal cavity and the cranial cavity. The hypophysis could not be made out. The tumor was connected, however, with the infundibulum of the hypophysis. Histologically, it consisted of connective tissue, glia fibers, which contained pigment, bone, cartilage, blood vessels, fat tissue, smooth muscle fibers, different types of epithelium, nervous tissue and embryonal tissues. Besides the tumor, autopsy revealed an atrophy of the thymus and both suprarenals. The author believes that this tumor should be classified among teratomas rather than as epignathus because of the location of the tumor, because of the absence of any parts of well developed organs, such as extremities, and because of the tumor-like growth. The relation of the tumor to the hypophysis was not studied because the hypophysis could not be located.

O. SAPHIR.

HAMARTOMAS OF THE INTRAHEPATIC BILE DUCTS AND THEIR RELATIONS TO CYSTIC LIVER. W. AUERBACH, Frankfurt. Ztschr. f. Path. **40**:272, 1930.

The liver of a 69 year old woman contained many green and brownish-green nodules, not exceeding 8 mm. in diameter. The nodules showed a smooth cut surface and were well circumscribed. Histologically, small cysts were found embedded in a dense connective tissue which was poor in nuclei. The cysts were lined by one row of high cylindric cells and contained an amorphous greenish-brown material. A basal membrane was not clearly distinguishable. The wider the cysts, the more flattened was the lining epithelium and the more did the lining cells resemble endothelial cells. A few cystic portions were actually lined by endothelial cells. These cysts were filled with red blood cells and resembled structures found in hemangioma. No connections could be made out between the cysts and the adjacent bile ducts. The author classifies these cysts as hamartomas which for some reason did not develop fully into a cystic liver, but remained stationary.

O. SAPHIR.

TUMORS OF THE NEUROMYO-ARTERIAL GLOMUS (MASSON). M. HOPF, Frankfurt. Ztschr. f. Path. **40**:387, 1930.

The neuromyo-arterial glomus, which was described by Masson as an organ that regulates the temperature and blood pressure, sometimes gives rise to a small benign tumor called angioneuromyoma. This tumor formerly was called angiosarcoma, endothelioma, etc. Clinically, this growth is characterized by a history of trauma, slow growth and intense pain. It is found in the region of the extremities. The tumor is soft, measuring up to 2 cm. in diameter, bluish red, and somewhat resembling an angioma. It is always well circumscribed. Microscopically, there are many vessels, the walls of which consist of epithelioid cells, smooth muscle fibers and nonmedullated nerve fibers. The tumor is surrounded by a connective tissue capsule that distinguishes this type of tumor from an angioma. The author reports four such cases.

O. SAPHIR.

PRIMARY SARCOMA OF THE PERICARDIUM. G. BODON, Frankfurt. Ztschr. f. Path. Path. **40**:417, 1930.

Carcinoid of the skin, squamous cell carcinoma of the bronchus, hypernephroma of the right kidney and osteochondroma of the lung are reported in a man aged 89.

O. SAPHIR.

PRIMARY SARCOMA OF THE PERICARDIUM. G. BODON, Frankfurt. Ztschr. f. Path. **40**:431, 1930.

Primary sarcoma of the pericardium is reported in a 65 year old man. Histologically, the tumor consisted of round cells of the size of lymphoblasts which diffusely infiltrated the pericardium. The cells showed large, round, dark-stained nuclei which were surrounded by a small mass of cytoplasm. Many nuclei showed a crescent shape and indentations which were taken as various forms of amitotic division. Between these cells, a fine reticulum was noted, which surrounded, in some fields, individual cells. There were many vessels found. The tumor cells invaded the myocardium and the subepicardial fat tissue. The case showed, in addition, a meningioma.

O. SAPHIR.

THE HISTOLOGIC CHANGES IN CARCINOMATOUS OSTEOMALACIA. K. MAKRYOCOSTAS, Frankfurt. Ztschr. f. Path. **40**:501, 1930.

The author reports a case of a primary carcinoma of the uterus in a woman 79 years of age, with metastases to both femurs, both tibiae, fibulae and the metatarses. Special attention is given to the histologic lesions of the bones which are described in greatest detail. Also the synovial changes are carefully studied. The bone and spongiosa were destroyed first, while in some instances the cartilaginous articular end-portions were preserved, loosely attached to the underlying carcinomatous tissue which had completely replaced the osseous portions. Eventually, the cartilaginous end-portions were also replaced by tumor tissue.

O. SAPHIR.

MALIGNANT CYSTADENOMAS OF THE LIVER. I. TOUSSAINT, Frankfurt. Ztschr. f. Path. **40**:538, 1930.

Two cases are reported. In both, carcinomatous portions were found in addition to structures of benign adenomas with large spaces justifying the term "cysts." The author believes that only one similar case is reported in the literature. This is the case of R. Jaffe (*Frankfurt. Ztschr. f. Path.* **21**:26, 1918), who found such a tumor in the liver of a dog. It is thought that this tumor arises from bile ducts on the basis of a developmental disturbance.

O. SAPHIR.

CANCER AND LIPOID METABOLISM. F. BURGHEIM and W. JOEL, Klin. Wchnschr. **10**:397, 1931.

The cholesterol content of malignant tumors is always greater than in benign tumors, and there may be a relative increase in cholesterol in the vicinity of cancers. This increase may occur in the precancerous stage. The importance these results may have in the microscopic diagnosis of cancer is emphasized.

COMPLEMENT FIXATION WITH ALCOHOLIC CARCINOMA EXTRACTS. M. FLOKSZTRUMPF and J. KOŁODZIEJSKI, Klin. Wchnschr. **10**:1120, 1931.

The theoretical postulates of the Hirszfeld and Halber carcinoma reaction rest on the following observations: (1) the serums of rabbits immunized with carcinoma extracts react selectively with alcoholic extracts of carcinoma, and (2) the serum of patients with carcinoma binds complement with cholesterolized alcoholic extracts of carcinoma, but not with similar extracts of normal tissues. A total of 475 carcinoma serums and 2,406 control serums were tested. The percentage of positive reactions in carcinoma varied between 0 to 80 according to the origin of the primary growth and the clinical progress of the disease. Early cases of carcinoma were not detected by this method. Visceral carcinoma, especially when disseminated, was detected in 80 per cent, skin carcinomas rarely. In cases of rapidly fatal carcinoma positive reactions rarely occurred. The serums that gave

positive Wassermann reactions and those of pregnant women among the controls reacted positively with the cancer antigen. Patients with hyperthyroidism often reacted positively. Thus 4 to 5 per cent of the control serums reacted positively. These anomalous reactions are without explanation.

EDWIN F. HIRSCH.

Technical

NEW TECHNIC AND INSTRUMENT FOR OBTAINING BIOPSY SPECIMENS. WILLIAM J. HOFFMAN, Am. J. Cancer **15**:212, 1931.

A new technic and instrument are presented for obtaining specimens for biopsy.

B. M. FRIED.

STANDARDIZATION OF METHOD BASED ON MESOBILIRUBINOGEN (H. FISCHER) FOR ESTIMATION OF UROBILINOGEN. C. J. WATSON, Arch. Int. Med. **47**:698, 1931.

A modification of Terwen's method for the estimation of urobilinogen in urine and feces is described, which is believed to be simpler and more efficient than the original procedure. The standardization of the color standard is based on crystalline mesobilirubinogen (Fischer). The results of a study of a series of cases with this method are reported. They indicate that valuable information may be obtained by its use in the clinical study of jaundice and the anemias. Persistent urobilinuria after the reticulocyte crisis in pernicious anemia in cases in which liver therapy is used is accompanied by a slower rate of increase of hemoglobin and, to a less marked degree, of erythrocytes.

AUTHOR'S SUMMARY.

SERUM AND PLASMA BILIRUBIN: A COMPARATIVE QUANTITATIVE STUDY OF ONE HUNDRED CASES. MENDEL JACOBI, REUBEN FINKELSTEIN and RUDOLPH KURLEN, Arch. Int. Med. **47**:759, 1931.

The results of simultaneous quantitative determinations of serum and plasma bilirubin by the Thannhauser and Andersen modification of the van den Bergh technic in 100 consecutive and unselected cases are reported, and the results are analyzed as to the degree of agreement or disagreement. The readings are shown to correspond in a very high degree, and they indicate that plasma instead of serum may be used with equal accuracy in determinations of bilirubin. The results confirm the statements of McNee and Keefer. They are directly opposed to the figures reported by Shay and Schloss from eleven observations with wide discrepancies in the readings. An increase in the length of time allowed for coupling up to one hour apparently plays no part in the slight quantitative differences noted.

AUTHORS' SUMMARY.

MODIFIED ASCHHEIM-ZONDEK TEST FOR PREGNANCY. F. EBERSON and M. H. SILVERBERG, J. A. M. A. **96**:2176, 1931.

The modification consists in injecting into immature female rats of the same litter, from 18 to 21 days old, a suspension of a precipitate obtained by adding alcohol to the urine. By injecting 1 cc. of the test material twice a day for one or two days, the time required to reach a diagnosis has been reduced to from thirty-six to forty-eight hours. When the test is positive, the ovaries, tubes and uterus are enlarged; the tubes are distended, and the ovaries are hemorrhagic and congested, with protruding follicles and "blood points." "In the absence of macroscopic changes in the ovaries, the microscopic observations of a peripheral organization of follicular cells and a peculiar vitreous alteration of the central mass of cells is diagnostic of very early pregnancy or of its arrest. Despite the absence of luteinization, these structural and tinctorial changes noted in the follicular cells

are specific and especially valuable in conditions that preclude diagnosis from the macroscopic observations alone." This method has given uniformly satisfactory results.

NEW LITHIUM SELECTIVE AND ENRICHMENT METHODS FOR THE ISOLATION OF SALMONELLA ORGANISMS. J. D. A. GRAY, J. Path. & Bact. **34**:335, 1931.

Staphylococcus, Bacillus foecalis-alkaligenes and those members of the Salmonella group concerned with human disease are markedly resistant to the salts of lithium (chloride and carbonate). For a selective method for the isolation of members of the Salmonella group, the addition of 2, 2.5 and 3 cc. of 10 per cent aqueous solution of lithium chloride to plates containing 15 cc. of MacConkey's medium is recommended. Forty-eight hours' incubation is usually required. For an enrichment method for the isolation of members of the Salmonella group, the addition of 1, 2 and 3 cc. to 10 cc. of peptone water (p_H 7.6) is recommended. Incubation for twenty-four or for forty-eight hours before subinoculation on MacConkey's medium gives the best results. These selective and enrichment methods were found to give results for the isolation of members of the Salmonella group quite as good as, and usually better, than those obtained with the brilliant green enrichment method with or without the addition of telluric acid. Both MacConkey's medium and the peptone water containing lithium chloride may be kept ready for use. Little or no effect on the selective action of the lithium chloride was obtained by the presence of sodium chloride, brilliant green, telluric acid or brilliant green and telluric acid. Removal of the particulate organic matter in the fecal emulsions gave slightly better chances of isolating the Salmonella organisms.

AUTHOR'S SUMMARY.

NEW COLLODION MEMBRANES FOR BACTERIOLOGICAL USE. W. J. ELFORD, J. Path. & Bact. **34**:505, 1931.

A new series of graded ultrafilter membranes suitable for general bacteriologic use is described. The principles and technic of the preparation of these membranes are discussed in detail. A wide range in permeability is available, from 3 microns down to 10 micromicrons or less. The membranes have excellent uniformity.

AUTHOR'S SUMMARY.

ASBESTOSIS BODIES IN THE FAECES IN PULMONARY ASBESTOSIS. S. ROODHOUSE GLOYNE, Tubercle **12**:158, 1931.

As a result of an examination of feces for asbestosis bodies, the author believes this method, in the absence of findings in the sputum, forms an alternative, although admittedly a poor, substitute for clinching the diagnosis.

H. J. CORPER.

QUANTITATIVE DETERMINATION OF ACETONE WITH PULFRICH'S PHOTOMETER. C. URBACH, Biochem. Ztschr. **236**:164, 1931.

Urinary acetone is quantitatively determined with Pulfrich's photometer by use of the following technic: From 20 to 100 cc. of a twenty-four hour specimen of urine, kept on ice and well mixed, is put into a distillation flask and the flask filled up to 300 cc. with distilled water. Then 5 cc. of concentrated sulphuric acid is added, and the mixture is distilled into a flask containing 25 cc. of distilled water. After the distillate is filled up to 200 cc., 2 cc. of the following potassium hydroxide solution is added: solution 1, compound of 11.33 normal solution of potassium hydroxide, or 63.6 Gm. of potassium hydroxide in 100 cc. of water, and 1 cc. of salicylic aldehyde. (Solution 2: 10 per cent [Gm.] solution of salicylic aldehyde in 95 per cent alcohol.) The mixture is heated while being shaken over a water bath at 50 C. for exactly twenty minutes, till a red color develops. After

cooling, it is diluted to 15 cc. The distillation is not necessary if the urine does not contain any sugar. Pulfrich's photometer with filter S 53 or S 50 is used, the distillate being compared with a mixture of 10 cc. of solution 1 plus 20 cc. of solution 2 plus 120 cc. of water. After determination of the extinction coefficient, the amount of acetone can be read from a table of predetermined amounts of acetone.

WILHELM C. HUEPER.

THE VALUE OF THE PRECIPITATION TESTS FOR THE DIAGNOSIS OF SYPHILIS. F. E. HAAG and I. LINKWEILER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **70**:337, 1931.

The Precipitation Test as a Routine Procedure in the Serodiagnosis of Syphilis (I. Linkweiler).—One thousand serums were tested in the Hygienic Institute in Düsseldorf with three complement-fixation and five precipitation tests: (1) the original Wassermann test (syphilitic liver extract), (2) citochol extract, (3) Bordet extract, (4) the Meinicke test, (5) the Kahn test (original method), (6) the Kahn test with citochol extract, (7) the citochol test (original method) and (8) the Sachs-Georgi test with citochol extract. All tests agreed in 93.5 per cent. Only 1.3 per cent of the cases showed absolute disagreement, the disagreement of the balance being due to qualitative differences. The citochol extract proved particularly sensitive.

The Practical Significance of Positive Precipitation Tests in the Presence of Negative Complement-Fixation Tests (F. E. Haag).—The results of six comparative tests on 11,345 serums and spinal fluids are reported. The aforementioned three complement-fixation tests and the following three precipitation tests were used: (4) the Kahn-citochol reaction, (5) the Meinicke test and (6) the Müller-Ballungs test. Agreement was found in 94 per cent and absolute disagreement in 1.1 per cent of the cases. The precipitation tests proved more sensitive in old cases in which treatment had been given. The Kahn test with the citochol antigen showed the highest sensitiveness and specificity. In 460 old cases in which treatment had been given, the complement-fixation was negative 280 times, and precipitation only 14 times.

I. DAVIDSOHN.

A METHOD FOR COUNTING DEAD AND LIVING TUBERCLE BACILLI IN THE SPUTUM. E. BUTSCHOWITZ, Ztschr. f. Tuberk. **60**:149, 1931.

Tubercle bacilli are counted in smears made from digested sputum to which a known number of yeast cells have been added. The sputum is cultured at the same time. The author believes that by comparing the number of microscopically demonstrable tubercle bacilli with the number of colonies the ratio of dead and living bacilli can be determined.

MAX PINNER.

Book Reviews

A COLLECTIVE REVIEW OF RECENT BOOKS ON CLINICAL PATHOLOGY*

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ROCHESTER, MINN.

Clinical pathology has developed into a true medical specialty in the last quarter of a century. No greater evidence of this is necessary than the considerable amount of literature that is devoted to this field. If one were to include European literature, there would be no less than thirty-five texts of various kinds that have appeared in the last three years. I shall not attempt, however, to review any books except those that are printed in English. Almost all of these are American books.

Mention might be made first of a new journal in this field, *The American Journal of Clinical Pathology,* which appeared in 1931, published by Williams and Wilkins Company and under the editorship of T. B. Magath. The first volume will number about five hundred pages. The journal is the official publication of the American Society of Clinical Pathologists, a society consisting of specialists in the field, who have devoted more than three years to clinical pathology.

Manual of Physical and Clinical Diagnosis. By Dr. Otto Seifert (late Professor of Medicine, Würzberg) and Dr. Friedrich Mueller (Professor of Medicine, II Medical Clinic, Munich). Authorized translation from the twenty-fourth German edition by E. Cowles Andrus, M.D. (Associate in Medicine, Johns Hopkins University; Associate Physician, Johns Hopkins Hospital). Price, $6.00. Pages, 543 + xi, with 140 illustrations and three colored inserts. Philadelphia and London: J. B. Lippincott Company.

The book of Seifert and Mueller, is not, strictly speaking, on clinical pathology alone, but there is so much laboratory information in it and it is such a useful handbook that it is well worth mentioning. It is in its twenty-fourth edition in German. From this edition, we have the first American translation, by Andrus. The original preparation of this manual in German was prompted by Prof. C. Gerhardt. The data presented, on account of their quantity and diversity, can only with difficulty be accurately memorized. On the other hand, they are distributed in so many textbooks and monographs that it is tedious and time-consuming to hunt them out on each occasion. George E. Fahr of the University of Minnesota, representative of the author, presents an introductory preface in which he says: "Friedrich Mueller needs no recommendation from anyone. The twenty-four editions in the German language and the numerous editions in seven other languages attest to the value of this compendium of diagnostic methods and data. Every interne and clinical clerk should carry a copy in his white coat to be available when working on the wards or in the clinical laboratory. The student who has had a course of lectures on physical diagnosis and on clinical microscopy and chemistry will find that this little book is all that he will usually need to consult in his work on the wards. The practitioner of medicine

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* From the Section on Clinical Pathology, the Mayo Clinic.

who has a copy on his desk will only rarely have to consult any other work on diagnostic methods and data." The book may be called a true *vade mecum*. The translator states in the preface that "certain additions have been made to include procedures to which the American student and physician are accustomed: blood chemistry, staining methods, etc."

There are eleven chapters and an appendix. The eleven chapters are arranged as follows: introduction; respiratory organs; circulatory organs; the blood; urinary system; gastro-intestinal tract; parasites and infectious diseases; nervous system; the glands of internal secretion; metabolism and nutrition; diseases of the skin. The appendix contains a tabular summary of the symptoms and the therapy of acute intoxications, mineral springs and baths, drugs and dosage, and troy weights and metric equivalents. There are three colored inserts, one of these being double and presenting excellent illustrations of various types of blood cells. The clinical laboratory material is of course brief as regards actual technic, and the book is not designed as a laboratory manual, but it will be found useful as a reference book for the clinical pathologist in discussing clinical diagnosis with the clinician and will also be informative for the clinician in evaluating the reports of the clinical pathologist. The thin paper edition makes the book convenient in size, so that it can be readily carried in the overcoat pocket or in the physician's handbag.

Bedside Interpretation of Laboratory Findings. By Michael G. Wohl, M.D. (Associate Professor of Experimental Medicine, Temple University Medical School; Chief of the Metabolic Clinic, Temple University Hospital; Chief of the First Medical Diagnostic Clinic, Mount Sinai Hospital, Philadelphia, Pa.). With an introduction by Joseph McFarland, M.D., Sc.D. (Professor of Pathology, University of Pennsylvania). Price, $6.00. Pages, 321, illustrated. St. Louis: The C. V. Mosby Company, 1931.

Wohl states in his preface that the purpose of the volume is to deal with the middle ground between the fields of activity of the clinical pathologist and the general practitioner. Simple tests that can be carried out by the practitioner in his office are given in detail. The proper evaluation of the more complicated tests carried out by the clinical pathologist is presented for the use of the clinician in making the diagnosis. The introduction by McFarland contains some timely comments on the present-day physician's knowledge of laboratory procedures and his attitude toward "reports."

The twenty-three chapters of the text deal with the clinical interpretation of various tests under the following headings: sputum; gastric contents; duodenal contents; feces; blood; blood chemistry; blood cultures; blood groupings; acidosis and alkalosis; basal metabolism; urine; kidney functional tests; liver functional tests; cerebrospinal fluids; transudates and exudates; immunologic reactions; skin reactions; mouth and pharynx; eye and ear; skin (really a chapter on mycology); infections of the urethra and external genitalia; laboratory tests for pregnancy; miscellaneous tests. It will be seen from the diversity of subjects that the euphonious title of the book is somewhat of a misnomer, as in a large proportion of instances the interpretation of the laboratory results are not made at the bedside. The clinician, however, by using this book in his study, may gain the necessary information for an intelligent discussion of his case with the clinical pathologist, who should act as a consultant. On the other hand, the clinical pathologist should find this book a valuable adjunct in such consultations by using the concise information that is here given concerning the clinical interpretation of laboratory data. The selection of material has been made by the author from his extensive and valuable experience as a clinical pathologist. The chapter on laboratory tests for pregnancy by Mazer and Hoffman is of interest because of the description of the test for the female sex hormone originally described by these authors, using spayed mice as the test animals. It is regrettable, however, that mention is not made of the simple Friedman test, which is rapidly gaining in favor. This book should make good reading for the busy physician who wishes to keep up to date on diagnostic methods.

The Clinical Interpretation of Blood Examinations. By Robert A. Kilduffe, A.M., M.D. (Director of Laboratories, Atlantic City Hospital, and City Bacteriologist, Atlantic City, N. J.). Price, $6.50. Pages, 629, illustrated. Philadelphia: Lea & Febiger, 1931.

This is a more pretentious book than the one just reviewed, but in a narrower field. Kilduffe has demonstrated on many occasions his ability to state clearly the technic employed in various clinical laboratory procedures and the interpretations of the results. This new volume is an expansion of a previous compendium entitled "The Clinical Interpretation of the Wassermann Reaction." This volume contains thirteen chapters. The first chapter, on the physical properties of the blood, is clearly and ably written. This is followed by chapters on: blood formation and blood destruction; cytologic blood examination; clinical study of the cellular elements of the blood; the leukocytes; the blood platelets; the symptomatic blood picture in disease; laboratory studies of anemias; bacteriologic examination of the blood; serologic examination of the blood; the parasitology of the blood; the complement-fixation test; chemical examination of the blood. There are numerous references to the literature in footnotes, and the book will undoubtedly furnish a valuable guide to both the clinician and the laboratory worker in further study on the immense number of subjects that are treated. The technic is not given in detail, except in some of the simple procedures; it is not intended to be a laboratory manual. Probably the best chapter is the twelfth, on complement-fixation tests. In this field the author is well known, and he has put more of his personal experience into the writing of this chapter. In the final chapter, the subject of blood chemistry is as adequately handled as can be expected in the space alloted, about 125 pages. There is little to criticize unless it is that the author has included many unnecessary references to valueless tests. The author states in his preface that he has done this deliberately, feeling that it is due to the reader to have an evaluation put on the unsatisfactory, as well as on the valuable, diagnostic procedures. However, in reviewing such a collection, the reader becomes weary of so much that is "nonspecific" or of "little value." This is especially noticeable in the tenth chapter, in which ten pages, including comment on more than ten tests, are given to serologic studies in malignancy, all of no clinical value. In the same chapter, seven pages are devoted to unsatisfactory laboratory tests for the diagnosis of pregnancy, although every clinical pathologist is more or less familiar with the satisfactory Aschheim-Zondek test or the simple Friedman test. One wonders, in the consideration of agglutination tests, why Maniolow's test for the determination of sex is included, whereas the useful agglutination tests for diagnosing undulant fever are omitted, and only one and a half lines are devoted to the subject of tularemia. In the chapter on the symptomatic blood picture in disease, the various disorders are arranged alphabetically. Such an arrangement is natural enough, although it is somewhat disconcerting to find cholera, fractures, hay fever and heart disease considered in the order named. Although these minor criticisms are mentioned, on the whole the book will undoubtedly be found useful in the library of every clinician who has not been able to keep abreast of the vast amount of work that has been done in recent years in the field of examinations of the blood.

Quantitative Clinical Chemistry. By John P. Peters, M.D., M.A. (Professor of Internal Medicine, Yale University School of Medicine) and Donald D. Van Slyke, Ph.D., Sc.D. (Member of the Rockefeller Institute for Medical Research). Vol. I: Interpretations. Price, $12.00. Pages, 1264 + xvi. Baltimore: Williams & Wilkins Company, 1931.

Peters and Van Slyke's book on interpretations of laboratory procedures will doubtless last for many years as the best example of such writing. The first volume has just appeared and bears a subtitle "Interpretations." These authors have prepared a monumental work. If the second volume, which is to be on technic, is as complete as this volume on interpretations, it is safe to say that it will be the authoritative work in English on the subject of clinical chemistry.

There are 3,890 references to the literature in this volume alone, which make it exceedingly valuable if considered only for this useful feature. The style is clear and readable for the clinician or laboratory worker who is not entirely conversant with all of the technical procedures considered. The reputation of the authors in the scientific world for exact evaluation of scientific data is upheld in every particular. The chapters are on: total metabolism; carbohydrates; lipoids; nonprotein nitrogen and nitrogen metabolism; urine, ammonia; amino-acids; uric acid; creatine and creatinine; total organic acids; lactic acids and ketones; phenols; hemoglobin; proteins of the blood plasma, urine and other body fluids; blood volume; total base, sodium and potassium; calcium, magnesium; carbonic acid and acid-base balance; chlorides, phosphorus and sulphur. We shall await with much interest the appearance of the second volume.

Chemical Methods in Clinical Medicine. Their Application and Interpretation with the Technique of the Simpler Tests. By G. A. Harrison, B.A., M.D., B.Ch. (Cantab.), M.R.C.S. (Eng.), L.R.C.P. (Lond.) (Reader in Chemical Pathology, University of London; Reader and Lecturer on Chemical Pathology, St. Bartholomew's Medical College; Chemical Pathologist, St. Bartholomew's Hospital; Formerly Chemical Pathologist, King's College Hospital and Hospital for Sick Children, Great Ormond St., London). Price, $5.25. Pages, 534 + ix, with two color plates and 63 illustrations. New York: The Macmillan Company, 1930.

Harrison's book was printed in Great Britain, but was distributed in this country by the Macmillan Company. It contains twenty-six chapters, an appendix and a well arranged index. The introductory chapters are: definition of terms; preparation of standard solutions; description of apparatus. The next fifteen chapters are on urinalysis; in fact, the author makes much over the point that unfortunately in recent years interest has waned in this important field of clinical chemistry owing to the overshadowing attention that has been paid to chemistry of the blood. Some of the chapters, to be sure, are brief. They are listed as follows: routine qualitative tests; albuminuria, both qualitative and quantitative tests; urinary deposits; tests of renal efficiency, with a very good, detailed description of the Maclean-de Wesselow test (urea-concentration test); reducing substances in the urine, blood sugar curves; chemical tests in diabetes mellitus and the control of insulin treatment; ketosis, acidosis and alkalosis, clinical significance and technical methods; blood and its derivatives in the urine, the direct vision spectroscope; urine abnormal in color, with special reference to drugs in the urine; bile and urobilin in the urine, with efficiency tests of the liver and bile passages; tests of pancreatic efficiency; indicanuria, lipuria and skatole-red, diazo-test, chyluria, nitrituria; chlorides, and inorganic constituents of the urine; the collection, preservation and quantitative analysis of the urine. There are three chapters on blood: the collection and preservation of blood; qualitative examination, in which the "formol-gel test" and Jaffe's test for indicanemia are considered; chemical analysis of the blood. The last seven chapters are devoted to: chemical examination of the cerebrospinal fluid; analysis of milk; gastric analysis; chemical examination of duodenal contents; chemical examination of feces; basal metabolism and metabolism experiments; "miscellanea and conundrums." The last chapter tells among other things how to determine whether the urine is contaminated with feces or whether a laparotomy wound contains pancreatic juice. As these and similar questions occasionally tax the ingenuity of the clinical pathologist, the author's solutions make interesting reading. The appendix also contains a number of useful tables and miscellaneous information.

Harrison pays particular attention to calculations. Seldom is each step in the mathematic computation of a test elaborated in such detail. This is deliberately done by the author, as he stated in the preface: "That the calculations are given very fully—some readers may consider in absurd detail. . . . In the author's experience more mistakes are made in the calculations than in the actual technic by the average student and technical assistant and also because in his opinion many writers give calculations too briefly."

For the most part this book will prove interesting and instructive to American readers. The simple illustrations of the line-drawing type are useful in clarifying technical procedures. One criticism may be made of the manner of making cross-references. The reference is always merely to a chapter and not to the page or pages. The index, however, is complete and well arranged. There are also complete references to other texts and to the literature generally with each chapter.

Clinical Laboratory Methods. By Russell Landram Haden, M.A., M.D. (Professor of Experimental Medicine, University of Kansas School of Medicine, Kansas City, Kan.). Edition 3. Price, $6.75. Pages, 317, with 69 illustrations and four color plates. St. Louis: The C. V. Mosby Company, 1929.

Practical Clinical Laboratory Diagnosis Including the Interpretation of Laboratory Findings. Designed for the Use of Students and Practitioners of Medicine. By Charles C. Bass, M.D. (Dean and Professor of Experimental Medicine, School of Medicine, Tulane University of Louisiana) and Foster M. Johns, M.D. (Assistant Professor of Medicine and Director of the Laboratories of Clinical Medicine, the School of Medicine, Tulane University of Louisiana) Edition 3. Price, $7.50. Pages, 187 + xvi, with 134 black and white textual figures and 20 plates, nine of which are in color. Baltimore: Williams & Wilkins Company, 1929.

A Manual of Clinical Laboratory Methods. By Clyde Lottridge Cummer, PH.B., M.D., F.A.C.P. (formerly Associate Clinical Professor of Clinical Pathology, School of Medicine, Western Reserve University, Cleveland; Instructor in Dermatology and Syphilology, School of Medicine, Western Reserve University; Visiting Dermatologist, Charity and St. Alexis Hospitals, Cleveland). Edition 3, thoroughly revised. Price, $6.75. Pages, 583 + xx, with 173 engravings and 12 plates. Philadelphia: Lea & Febiger, 1931.

Clinical Diagnosis by Laboratory Methods. A Working Manual of Clinical Pathology. By James Campbell Todd, Ph.B., M.D. (late Professor of Clinical Pathology, University of Colorado, School of Medicine) and Arthur Hawley Sanford, A.M., M.D. (Professor of Clinical Pathology, University of Minnesota [Mayo Foundation]; Head of Section on Clinical Laboratories, Mayo Clinic, Rochester, Minn.) edition 7, thoroughly revised. Price, $6.00. Pages, 765, with 347 illustrations, 29 in colors. Philadelphia: W. B. Saunders Company, 1931.

Within the last three years there have been new editions of these four of the leading texts. Although it may not seem necessary to give a complete description of each of these well known books, it would seem best to comment on them in this review, since the scope of each one of these works may not be known to all readers.

Haden's book appeared in its third edition in 1929. The book was thoroughly revised and brought up to date. It is not a textbook; the author has rather presented his notes in the form that he has found useful in the laboratory of a general hospital. His wide experience in medicine and in teaching has enabled him to make a useful selection of those diagnostic procedures that he believes are most worthwhile. There is no attempt to explain results. Normal values are often given, however. In fact, among the chief features of the book are several tables in which normal and abnormal data are listed. One of the most interesting of these is the first table in the book on the identification of reducing substances in the urine. By carrying out half a dozen tests in the order listed, various reducing substances, as well as dextrose, may be identified. Another convenient and unique feature of the book is the placing of the reference to the original article immediately below the title for the text. There is no hunting in a bibliography at the end of the book or even in a footnote for the reference to the literature.

There are fourteen chapters, arranged as follows: qualitative examination of urine; quantitative chemical examination of urine; analysis of gastric juice; examination of sputum; examination of feces; qualitative examination of the blood; quantitative chemical examination of the blood; serologic technic; preparation of bacteriologic solutions; stains and mediums; general bacteriologic methods; miscellaneous clinical pathologic examinations; miscellaneous chemical procedures and solutions; histologic technic; examination of milk and water. A thoughtful study of the numerous tables and the brief descriptions of the various tests will commend this book to the laboratory worker and clinician alike. The diction is clear and concise. The nomenclature in the consideration of animal parasites and of bacteria is correct and accepted.

In 1929, also, appeared the third edition of Bass and Johns' book. This small book contains much interesting and original matter. It is chiefly a "picture book." The authors have evidently felt that accurate photographs illustrating the various steps of technical procedures would impress the student more readily than a word description. There are 134 of these illustrations in black and white, and twenty plates, nine of which are in colors, scattered through 179 pages of text. Some of these illustrations are well done, while others would seem to be unnecessary. They may emphasize certain laboratory rules for the medical student, but have no real part in the practice of clinical pathology by an experienced worker. The authors have a somewhat original method of arranging their text. There are seventeen chapters, many of them arranged in a manner found in most other books, but some of the chapters deal with only a single disease and the tests connected with the diagnosis of that condition. This is not strange when one remembers the interesting pathologic material in certain fields in New Orleans. The first five chapters are: use and care of the microscope; blood smears and stains; differential leukocyte counts; total counts in hemoglobin estimations; special tests of blood. The latter includes the determination of blood sugar, which is the only test having to do with the chemistry of the blood described in the book. The sixth chapter is brief and deals solely with malaria. Then follows chapters as follows: typhoid agglutination tests; urine; gastric contents; feces; pus, exudates and similar material; sputum. The chapter on feces is doubtless one of the best chapters in the book. The thirteenth chapter is one and a half pages in length and is on the laboratory diagnosis of leprosy. The last four chapters are on: spinal fluid; diphtheria; gonorrhea; syphilis. None of the various modifications of the Wassermann test is described, and the only serologic test that is given in detail, and that rather briefly, is the Butler precipitation test. Although this may be an excellent test, it is not as well known as the Kahn or the Kline test, of which no mention is made. Two appendixes contain the usual formulas and lists of recommended apparatus found in most textbooks.

The third edition of Cummer's well known manual appeared in 1931. The book is brought up to date in the twelve chapters and appendix, which are arranged as follows: examination of the blood; pathologic histology and differential diagnosis; parasitology and bacteriology of the blood; immunology; examination of chemical changes in the blood; urine; gastric and duodenal contents; feces; sputum; body fluids; basal metabolism; miscellaneous methods. The eleventh chapter, on basal metabolism, by M. A. Blankenhorn, includes a description of the Benedict method. The twelfth chapter is on bacteriologic methods. The appendix contains some valuable miscellaneous information. A scheme for the examination of a large number of specimens of urine in a hospital laboratory is described in considerable detail. The preparation of normal solutions is well presented. There are also a dozen similar subjects that reflect the experience of the author and that will appeal to the reader. A rather unique feature is the printing on the back fly-leaf and inside cover of a table of normal findings. However, it is unfortunate that the bibliography at the end of the book has not been brought up to date; this is particularly true of the textbooks. The reader is entitled to references to the latest editions. In many instances, several editions have appeared since the one that is referred to.

Probably the best chapter in the book is the fourth, on immunology. In the consideration of agglutination tests, both tularemia and undulant fever are described. The Kolmer-Wassermann technic is given fully, also a most interesting and complete description of the so-called Cleveland method, which was developed by a committee of various laboratory workers appointed by the Academy of Medicine of Cleveland, in the hope that a uniform technic would thereafter be used in Cleveland. Thus the test was named after the city in which it was developed. Both the Kline and the Kahn flocculation tests are given satisfactorily. Criticism must be made of the chapter on intestinal parasites. The choice of Dibothrycocephalus latus for the name of the broad Russian tapeworm is not now accepted by parasitologists, who prefer, on good and sufficient grounds, Diphyllobothrium latum. One is also somewhat amazed to read in the consideration of "examination for amoeba" that "the examination should be conducted at the bedside when possible." Is this ever done? The book is well illustrated and indexed, and the author is to be congratulated on this new edition, which includes much new material: for example, the silver impregnation methods for spirochetes, the Alzheimer method for studying the cellular contents of the spinal fluid, a description of cistern puncture, the alcohol test meal and the use of histamine in gastric analysis.

This year (1931) has also seen the appearance of the seventh edition of Todd and Sanford's book. This book probably needs no extensive review. The original author, the late Dr. Todd, was well known as a master of his subject. The joint author, to whom has fallen the task of bringing out the present and future editions, has endeavored to bring the book up to date. A few of the little used or obsolete tests have been omitted. The new material includes Corper and Uyei's method for the culture of bacteria of tuberculosis, Fairhall's method for the determination of lead, Folin's method (1929) for the precipitation of protein from blood and body fluids, his modified method for the determination of uric acid in the blood, and his revised copper solution for the determination of blood agar. Clark and Collip's method for the determination of calcium is given in full. The technic of the Keith, Rowntree and Geraghty method of determining blood volume and plasma volume is given in detail. The alcohol meal and the gastric reaction to histamine are considered in the chapter on analysis of gastric contents. The Gregerson test for occult blood is included. The Aschheim-Zondek test and the simpler Friedman test for pregnancy are briefly described. The chapter headings are: sputum; urine; blood; gastric and duodenal contents; feces; animal parasites; pus; puncture fluids, animal inoculation; miscellaneous examinations serodiagnostic methods; bacteriologic methods; vaccines and biologic skin tests. The paper used is thin, so that the book is larger than it appears, there being 765 pages. Some of the plates are becoming worn, and it is hoped that in future editions more attention may be paid to the illustrations.

Laboratory Medicine. A Guide for Students and Practitioners. By Daniel Nicholson, M.D. (Member of the Royal College of Physicians, London; Assistant Professor of Pathology, University of Manitoba; Assistant in Pathology, Winnipeg General Hospital). Price, $6.00. Pages, 437, with 108 engravings and a colored plate. Philadelphia: Lea & Febiger, 1930.

Nicholson's book has been thoroughly reviewed in THE ARCHIVES (**11**:168, 1931). The author has put into this his own experiences as a clinical pathologist. The result is pleasing, and the book has been well received.

A Textbook of Laboratory Diagnosis with Clinical Applications for Practitioners and Students. By Edwin E. Osgood, M.A., M.D. (Assistant Professor of Medicine and Biochemistry, and Director of Laboratories, University of Oregon School of Medicine) and Howard D. Haskins, M.D. (Professor of Biochemistry, University of Oregon School of Medicine, Portland, Ore.). Price, $5.00. Pages, 475 + xix, with 21 figures and 6 colored plates. Philadelphia: P. Blakiston's Son & Company, 1931.

Osgood and Haskins embody, in pleasing form, their lecture notes as presented to their students. The authors are both well known, particularly in the

field of hematology and chemistry of the blood. Their book is divided into two parts. Part I is written from the point of view of the general practitioner, with the clinical value and the interpretation of certain laboratory procedures considered more or less fully in relation to various diseases and symptoms. The arrangement is original but not entirely logical. Disorders of the kidney and urinary tract with special reference to nephritis are treated first, and are naturally followed by a chapter on disorders of carbohydrate, protein and fat metabolism with special reference to diabetes mellitus and disturbances of acid-base equilibrium. Pregnancy and its complications next claim attention, followed by disorders of the central nervous system with emphasis on the differential diagnosis of coma. The remaining four chapters of part I are on: disorders of the gastro-intestinal tract; basal metabolism; hematology; respiratory and cardiac disorders.

Part II takes up fully the technical procedures in common use referred to in part I. The arrangement is satisfactory enough if the reader follows the suggestion that "this text is to be thought over thoroughly, not merely to be read or memorized by rote." The numerous references to the literature are carefully selected and should stimulate the student to much collateral reading. He will also find useful the well planned index of diseases, which is, however, "not a substitution for cerebration." The chemical procedures, as might be expected, are well presented. There are many modifications of well known tests that are introduced from the experience of the authors.

Not enough attention is paid to examination of the feces; only eight pages with one table and one plate in which the illustrations are not adequate are devoted to the subject. This is probably the weakest part of the book. The Aschheim-Zondek test for pregnancy is well presented, but no mention is made of the simpler Friedman test, which is probably as reliable. Hematology is considered in an interesting, instructive and stimulating manner. It is safe to say that the color plates, reproduced from the drawings by Miss Clarice Ashworth, are as fine as any illustrations of blood cells that have appeared in American publications. The printing as a whole and the general appearance of this first edition lead one heartily to congratulate the authors.

Approved Laboratory Technic, Clinical, Pathological, Bacteriological, Serological, Biochemical, Histological. Prepared under the auspices of The American Society of Clinical Pathologists. By John A. Kolmer, M.D., Dr. P.H., D.Sc., LL.D. (Professor of Pathology and Bacteriology, Graduate School of Medicine, University of Pennsylvania; Professor of Immunology and Chemotherapy, School of Medicine, Temple University; Head of the Department of Pathology and Bacteriology, Research Institute of Cutaneous Medicine) and Fred. Boerner, V.M.D. (Associate Professor of Bacteriology, Graduate School of Medicine, University of Pennsylvania), assisted by C. Zent Garber, A.B., M.D. (Associate in Pathology, Peking Union Medical College; formerly Associate Pathologist, Henry Ford Hospital, Detroit) and by committees of the American Society of Clinical Pathologists, composed of Dr. J. H. Black, Dr. H. J. Corper, Dr. A. G. Foord, Dr. A. S. Giardano, Dr. F. W. Hartman, Dr. P. Hillkowitz, Dr. R. A. Keilty, Dr. R. A. Kilduffe, Dr. K. M. Lynch, Dr. A. H. Sanford and Dr. F. E. Sondern. Price, $7.50. Pages, 663 + xxii, with 11 colored plates and 300 illustrations in the text. New York and London: D. Appleton & Company, 1931.

This book is one of the most pretentious books on laboratory technic that has been published in America. It was prepared under the auspices of the American Society of Clinical Pathologists. The authors were assisted in this work by Garber and committees of the society. The book is dedicated to Dr. George H. Meeker of the University of Pennsylvania and to the memory of Ward Burdick, founder and first secretary of the American Society of Clinical Pathologists.

The object is "to establish standards for the performance of various laboratory examinations, to promote the practice of medicine by a wider application of clinical laboratory methods to the diagnosis of disease and to encourage a closer coöperation between the practitioner and the clinical pathologist."

The technic of each method has been definitely approved by at least five members of the society, although it cannot be stated that the society as a whole has approved of the methods described. The importance of using accurate and reliable apparatus and reagents is stressed. Quantitative tests are given special emphasis whenever possible. There is also included a brief chapter on methods for the microscopic examination of tissues by W. C. MacCarty and W. L. A. Wellbrock.

The book is divided into five sections and contains thirty-seven chapters, several of them on unusual subjects. The sections are: general laboratory methods; clinical pathologic methods; bacteriologic methods; serologic methods; clinical methods.

The chapters in section I are: use and care of the microscope and methods of micrometry; housing, feeding, inoculating, bleeding and necropsy of animals, and diagnosis of animal diseases; suggestions for routine and special laboratory examinations; prevention and emergency treatment of laboratory accidents.

Section II contains chapters of the more usual descriptions of clinical laboratory procedures. There is a comprehensive chapter on examination of the blood, and chapters on: methods for the examination of urine; renal functional tests; sputum; gastric contents; bile and duodenal contents; tests of hepatic function; feces; exudates and transudates; cerebrospinal fluid.

Section III, on bacteriologic methods, contains nine chapters: methods for the collecting and handling of material for bacteriologic examination; preparation and sterilization of glassware; preparation of culture mediums; general bacteriologic methods; diagnostic bacteriologic methods; preparation of bacterial vaccines; bacteriologic examination of milk; bacteriologic examination of water; testing of disinfectants.

Section IV, on serologic methods, has five chapters: collection of blood and serum; agglutination tests; blood transfusion tests; complement-fixation tests for syphilis and bacterial diseases; precipitation tests for syphilis.

Section V is headed "Clinical Methods." I believe this is a typographical error, as certainly seven of the nine chapters in the section are on chemical methods. There are adequate descriptions of methods of colorimetry, nephelometry and scopometry; preparation of standard solutions; chemical examination of the blood; chemical examination of urine; determination of basal metabolic rate; chemical examination of milk and other foods; methods for toxicologic examination. The thirty-sixth chapter is on the diagnosis of early pregnancy, and the last chapter in the book is on the microscopic examination of tissues. There is a carefully prepared index.

The quality of the paper has added greatly to the beauty of the book, although it has doubtless added greatly to the bulk and somewhat to the price. As a result, the illustrations, which are practically all also "approved," and which are borrowed from other publications, stand out with a remarkable clarity often not found in their original presentation. Plate I represents blood cells stained with "Wilson's stain." This stain is not well known to hematologists, and unfortunately no reference can be found in the text to its use. In collecting material from so many other texts, the authors have committed a not uncommon error in references to the literature: the original authority is not always given correctly. For example, in the description of Wright's stain it is stated that "employing a buffer solution is also recommended by Giardano." Although it is true that Giardano

makes the recommendation, this solution was described several years ago by Haden and in his book credited to McJunkin.

The chapter on methods for the examination of feces contains much valuable information. However, criticism may be made of the use of capitals in the species names of the parasites. Also in the use of Dibothryocephalus latus instead of Diphyllobothrium latum as the name of the "fish tapeworm." All of these are minor faults that may creep into any first edition. The beautiful appearance of the book itself and the wealth of information that it contains should make this a popular reference work for the clinical pathologist.

Protozoan Parasites of the Alimentary Tract. By Kenneth M. Lynch, M.D. (Professor of Pathology, Medical College of the State of South Carolina, Charleston, S. C.). Price, $3.75. Pages, 258 + xvii. New York: The Macmillan Company, 1930.

Although not strictly speaking a work on clinical pathology, mention should be made of Lynch's monograph. The author, a well known pathologist, is perhaps best known for his many publications on protozoology, and especially for his studies of the genus Trichomonas. He stated in his preface, however, that the book "is not intended for protozoologists. To biologists it will be an unconventional book. It is purposely so. Technical details which tend to confuse and so lose the interest of those to whom they are of no concern are largely left out of consideration." And in the introduction is the following paragraph: "Protozoologists are not pathologists; few pathologists and other physicians know much about protozoa. Therefore, the errors common among protozoologists in evaluating disease when associated with the presence of protozoa and among physicians in estimating the significance of the finding of protozoa in a sick person." There are fourteen chapters. The first chapter is on the life of protozoa, and the second on dissemination and prevention of infection. Then follow five chapters on the amebas, and these in turn by four chapters on the flagellates. The three remaining chapters are on the ciliates, the coccidia and the blastocystis. Although the book shows evidence of rather hasty preparation, it is an excellent compilation of the author's own views on the subject and should be very useful to the clinical pathologist.

COMMENT

It has been necessary to outline somewhat tediously the contents of each of these new publications. However, it will be seen that no one of them can completely cover the field. Although there is, of course, much duplication of descriptions of more or less standard procedures, there is an interesting variety in the manner of presentation, so that in many laboratories and in all large libraries there will be found everyday use for all of these new books on clinical pathology.

La Culture des tissus en biologie expérimentale. By Émile C. Craciun, Maître de conférences à la Faculté de medécine de Bucarest. Preface du Professeur G. Roussy. Price, 55 francs. Pp. 442, with 72 illustrations. Paris: Masson & Cie, 1931.

The reader of this book is left with the impression that a method reputed to be technically very difficult and accessible only to a chosen few was absolved from its former exclusiveness and made available to the mere mortals of the species of laboratory investigators.

The thoroughness and lucidity of presentation and the emphasis laid on minute details, so important in this field, deserve commendation. It will help to gain friends among those laboratory workers who are deterred from approaching this method of investigation by the exaggerated conception of its technical difficulties. Besides the sixty-four pages given to the discussion of general technical details, numerous additional specialized technical procedures are found all through the book. All varieties and modifications of technic are given in a convincingly objective manner, with a recommendation of those that proved best in the author's experience.

The study of tissues in vitro became an indispensable method of research for investigators in various disciplines. The general biologist, the zoologist, the botanist, the cytologist, the physiologist, the pharmacologist, the experimental radiologist, the pathologist, the bacteriologist and the immunologist use the technic with great profit, and all are undoubtedly interested in the results achieved with the tissue culture method in their respective fields. These achievements are presented in chapters 2 to 10, with exhaustive references to the original publications. The bibliography of fifty-seven pages appears to be complete.

The value of this excellent book could have been enhanced by the use of a paper of better quality and by good binding. As it is now, it will show quickly the wear and tear, particularly since, by its very nature, it is destined to be used on the laboratory table.

In the reviewer's opinion, the book merits translation to make it accessible to those who do not read French.

Books Received

A Manual of Clinical Laboratory Methods. By Clyde Lottridge Cummer, Ph.B., M.D., F.A.C.P., Instructor in Dermatology and Syphilology, School of Medicine, Western Reserve University. Third edition. Cloth. Price, $6.75. Pp. 585, with 185 illustrations. Philadelphia: Lea & Febiger, 1931.

Bulletin of the National Research Council No. 83. A Compendium of the Statute Law of Coroners and Medical Examiners in the United States. By George H. Weinmann. Issued under the Auspices of the Committee on Medicolegal Problems National Research Council. Price, $3. Pp. 240. Washington, D. C.: The National Research Council of the National Academy of Sciences, 1931.

Travaux des Instituts d'anatomie pathologique des Universités de Pologne. Editors, S. Ciechanowski (Kraków), W. Nowicki (Lwów), K. Opoczyński (Wilno), L. Paszkiewicz (Warszawa) and L. Skubiszewski (Poznan). Volume 2, nos. 3 and 4. 1931.

The Rockefeller Institute for Medical Research. History, Organization, Present Scope of the Scientific Work, Buildings and Equipment, Publications. Pp. 36. New York City; 1931.

Proceedings of the Pathological Society of Philadelphia. New series, volume 30; old series, volume 48. Containing the Transactions of the Society from January, 1927, to January, 1931. Philadelphia: Isolde T. Zeckwer, M.D., Recorder of the Society, 1931.

Anatomie pathologique. Par Maurice Letulle, professeur honoraire à la Faculté de Médecine de Paris. Avec la collaboration de L. Nattan-Larrier, professeur au Collège de France, et A. Jacquelin, médecin des Hôpitaux de Paris, L. Duclos, ophthalmologiste adjoint de l'Hôpital Chirurgical Gouin, E.-P. Normand, conservateur du musée d'anatomie pathologique générale Maurice-Letulle (Hôpital Boucicaut). Tomes I, II et III. Cloth. Price, 600 francs per set. Pp. 2346, with 843 illustrations. Paris: Masson & Cie, 1931.

Studies of Protection Against Tuberculosis: Results with B. C. G. Vaccine in Monkeys. By A. Stanley Griffith. Medical Research Council, Special Report Series, No. 152. Paper. Price, 9 pence. Pp. 49. London: His Majesty's Stationery Office, 1931.

Report of the National Research Council for the Year July 1, 1929-June 30, 1930. Established in 1916 at the request of the President of the United States under the charter of the National Academy of Sciences. Paper. Price, 15 cents. Pp. 119. Washington, D. C.: Supt. of Doc., Government Printing Office, 1931.

Resistance to Infectious Diseases: An Exposition of the Biological Phenomena Underlying the Occurrence of Infection and the Recovery of the Animal Body from Infectious Disease, with a Consideration of the Principles Underlying Specific Diagnosis and Therapeutic Measures. By Hans Zinsser, M.D., Professor of Bacteriology and Immunology, Medical School, Harvard University. Fourth edition. Cloth. Price, $7. Pp. 651, with illustrations. New York: The Macmillan Company, 1931.

Surgical Pathology of the Diseases of Bones. By Arthur E. Hertzler, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kan., Professor of Surgery, University of Kansas. Pp. 272, with 211 illustrations. Philadelphia: J. B. Lippincott Company, 1931.